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# Bulletin

*of the*

# Torrey Botanical Club

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# Fertilization in the incompatible cross Datura Stramonium × D. Metel

SOPHIA SATINA AND A. F. BLAKESLEE

(WITH PLATES 17 AND 18)

Crossability between species of *Datura* has been a subject of study for some years (Blakeslee, 1933). All the herbaceous species of *Datura* in our collection except *D. ceratocaula* and *D. Metel*, have given viable seeds in some combination with at least one other species. There is, however, considerable variation in the number of successful crosses among them. Successful reciprocal crosses are rare.

Although a cross between two species may not produce viable seeds, the foreign pollen nevertheless may serve as a stimulus for initiating growth of the capsule. Such capsules at maturity may contain only minute dried ovules or small aborted seeds which actually consist of seed coats only.

Inability to obtain viable seeds from some combinations is attributable to various causes. These may be classified into three groups: (1) inability of the pollen tubes to function normally in a foreign style; (2) failure of fertilization; (3) inability of the zygote or of the endosperm to develop, or arrest of their development before maturity.

A detailed discussion of our findings will be arranged according to the main stages in which abnormalities may be a cause preventing formation of viable hybrid seed. As a control, study was made of pollen grains and pollen tubes of *D. Metel* in *intra se* crosses and of *D. Stramonium* in *intra se* crosses. Furthermore, the whole process of fertilization and embryo development in *intra se* crosses of *D. Stramonium* was investigated.

## I. POLLEN GRAINS AND POLLEN TUBES

*Technique.* Methods used to study pollen grains, their germination and the pollen tube growth were as follows: (a) Smear preparations of pollen grains were fixed in Navashin's or in a modified Carnoy's fixative (Satina and Blakeslee, 1935) and stained by the Feulgen method. Details of this latter method are given by Margolina (1932). After treatment in normal or 0.5 normal solution of hydrochloric acid, pollen grains required staining in fuchsin sulphurous acid only ten to fifteen minutes instead of the one to three hours recommended by Margolina for other plant material. (b) For germination of pollen grains and early stages of pollen tube growth

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Trankowsky's (1931) method was found to be of great value. Pollen grains were sown on slides covered with a very thin layer of 1% agar with 2% to 12% saccharose. The slides were inverted on glass rods in large Petri dishes to which drops of water were added around the edge of the dishes. Moisture in Petri dishes is the most important factor for germination. To avoid bursting of the growing pollen tubes it was found necessary to keep the slides inverted. This prevents contact of young pollen tubes with water which would condense on the agar. The addition of a certain amount of sugar to Navashin's fixative (a slightly higher percentage of sugar than the one on which the pollen grains grew) prevents the bursting of pollen tubes when they are fixed. After being kept three to five minutes in such a mixture, the slides were placed in Navashin's solution alone. (c) For studying the pollen tube growth in the style, a number of flowers were castrated 24 hours before pollination and kept after pollination at 19.6 to 20°C. All these pollinations were made by Mr. L. F. Williams. At fixed intervals styles were dipped for one to two minutes into water at 60 to 62°C, then placed on a slide and dissected in aceto carmine with glass needles.

The microspore of *D. Metel* seems to develop in the same way as the microspore of *D. Stramonium*. The division of the nucleus in a young pollen grain (fig. 1) to form the tube and generative nuclei occurs before the pollen grain is fully mature. The size and affinity for stain of these nuclei differ considerably (fig. 2). The generative nucleus is smaller and easily found, due to its bright purple color when stained by the Feulgen method. It is cut off from the rest of the microspore by a membrane and forms a distinct cell at one side of the pollen grain. The larger round tube nucleus does not stain so intensely.

The germination of pollen grains can be studied either on the stigma or on the slides by the method just described. For cytological study the latter was preferred. The pollen grain of *D. Metel* begins to swell fifteen to twenty minutes after being sown on the agar slide and kept at 22 to 24°C. Thirty to forty minutes later, pollen tubes begin to grow through one of the pores of the pollen grain membrane. The tube nucleus which is ready to leave the pollen grain changes its shape. It is now oblong, vermiform, sometimes twisted and one of its ends is directed toward the pore (fig. 3). It escapes from the pollen grain before the generative nucleus which has remained almost unchanged in appearance up to this time. Two to four hours later both nuclei were found near each other in the pollen tube. The shape of both is very variable but they are more or less oblong. Further development of the pollen tube was studied in the styles. The generative nucleus of *D. Metel* when crossed *intra se*, as well as that of *D. Stramonium*, divides seven to nine hours after pollination to form the two

male gametes. Figure 4 shows the prophase stage of division of the generative nucleus in *D. Metel*. The division of the generative nucleus at the metaphase stage in the pollen tube of a selfed *D. Stramonium* is shown in figure 5. Twelve chromosomes are seen. The style was dissected and fixed nine hours after pollination. Ten to seventeen hours after pollination the tube nucleus still is closer to the tip of the pollen tube than the two male gametes. Sometimes the distance between the latter and the tube nucleus is very large, in other cases they are close together (fig. 6). The same is true of their distance from the tip of the pollen tube. The shape of the nuclei is also very variable: round or pear shaped, amoeboid or oblong. The position of nuclei in pollen tubes changes when the latter reach the micropyle (fig. 7). The tube nucleus then is found behind the male gametes, the latter are nearer to the tip of the pollen tube. Figure 7 shows part of a section made from an ovule of a control in *D. Stramonium* fixed 29 hours after pollination. The egg apparatus is ready for fertilization:—two synergids near the micropylar end of the embryo sac, the egg cell with the egg nucleus and a very large central nucleus formed from two fused polar nuclei.

The same behavior of nuclei in pollen tubes soon after pollination as well as in more advanced stages of development was found in crosses between *D. Stramonium*×*D. Metel*, except that the division of the generative nucleus took place 3 to 4 hours later. In the styles examined 23 hours after pollination, the length of the pollen tubes reached 5 to 6 cm. They were rather close to the ovule but still in the style. The pollen tube has two male gametes and one tube nucleus. The latter is still closer to the end of the pollen tube (fig. 8). As can be seen from the comparison of pollen tube growth in crosses and controls, no differences could be found except the rate of growth. The pollen tube in crosses grows more slowly than in controls.

## II. AND III. FERTILIZATION; THE PROEMBRYO AND ENDOSPERM DEVELOPMENT

A number of capsules of *D. Stramonium* used for control and 111 capsules of *D. Stramonium* pollinated with *D. Metel* were fixed in modified Carnoy (Satina and Blakeslee, 1935) or Navashin's solution. The pollinations were made in four different seasons (January, March, July, September), the first two having been made on greenhouse plants, the second two on field plants. Nearly a half of the 111 capsules were left on the plants to see whether seeds would be developed. Only a few capsules failed to set and dropped within seven to ten days after pollination. Cytological study

was made on 30 capsules which had been fixed at intervals from 22 hours to 8 days after pollination. Sections were cut from 5$\mu$ to 10$\mu$ in thickness. Seeds were removed from capsules and fixed from 8 to 25 days after pollination. These seeds were also imbedded in paraffin and sectioned. Free-hand sections were made of seeds which had been taken from capsules and fixed 25 to 40 days after pollination. All this material was stained with iron haematoxylin or by means of the Feulgen reaction. The latter was found to be of exceptional value for studying the fertilization process since it is specific for chromatin and leaves other substances in the cell unstained.

## II. FERTILIZATION

Guignard studied double fertilization in *D. laevis* in 1902. A detailed study of this process will be given in the present paper. Fertilization in both the cross and control flowers will be described together in order to avoid too much repetition. This process of fertilization seemed to be the same in both cases except for the time required for the pollen tube to reach the embryo sac. Whereas in the cross *D. Stramonium*×*D. Stramonium* the first few pollen tubes entered the micropylar end of the embryo sac 22 to 24 hours after pollination; in the cross *D. Stramonium*×*D. Metel* the first few pollen tubes apparently did not reach the same point until about 44 hours after pollination. In the latter cross no pollen tubes were found in the six ovaries which were fixed successively 19 to 27 hours after pollination. Of three ovules fixed 44 to 47 hours after pollination only one did not have pollen tubes. While it is not known definitely why pollen tubes were not found in these seven capsules it seems probable that they were fixed before the pollen tubes had reached the ovules. In all the other material examined 48 hours and more after pollination, there was only one ovary in which there was no evidence of pollen tube growth having taken place. This older material includes ovules and seeds from 31 capsules.

When a pollen tube reaches an embryo sac it passes through one of the synergids and ruptures in the embryo sac near the egg cell. The two male gametes together with a large amount of the cytoplasm from the pollen tube are released and the latter partly envelops the egg cell. Due to its strong affinity for iron haematoxylin, this mass could be observed during the whole process of fertilization and even later on near the young proembryo (figs. 16, 18, 19, 22, 24, 25). One of the male gametes approaches the egg nucleus, while the other approaches the central nucleus lying always close by (figs. 9, 10). Fusion of the egg nucleus with a male gamete rarely occurs at the same time as the fusion of the central nucleus with the other male gamete. It may precede or it may succeed the latter. Figure 11

shows an almost completed fusion of the male gamete with the egg nucleus, while the other male gamete had not yet come into contact with the central nucleus. This is true for both cross and control flowers. The shape of the male nucleus is slightly variable, but it seems that when it approaches the female nuclei it is usually round or pear-shaped (fig. 9 to 11). The clearest and best pictures of fertilization were obtained with the Feulgen reaction with which the cytoplasm from the pollen tube, and all other substances, except nuclei present in the embryo sac, remain colorless. (With such dyes as haematoxylin or crystal violet, however, they are strongly stained.) The double fertilization process could be watched step by step from the moment when the male gametes are released from the pollen tube to the moment when they have fused completely with the female nuclei. There is an obvious difference in the chromatic matter of the male and the female nuclei. The sperm nuclei are smaller, have larger chromatic particles and stain more deeply (figs. 9, 10, 11). Their dark purple color contrasts with the faintly stained egg and central nucleus, each of which contains a large stainless nucleolus (figs. 9–13). After a male nucleus has come in contact with a female nucleus, it elongates on the surface of the latter, then gradually is incorporated within it (figs. 11–13). It remains visible for a considerable length of time although its outline and area become increasingly vague. A very small and faintly stained nucleolus begins to form in the remains of the male nucleus (fig. 11). Toward the end of the fusion there remains a thin, oblong, more darkly stained area at the periphery of the egg and central nuclei (fig. 13). Fusion is considered complete when these nuclei stain uniformly throughout. Thereafter these nuclei may be called the zygote or fertilized egg and the endosperm nucleus respectively. The former is a diploid nucleus, the latter a triploid.

There seemed to be no visible difference between fertilization in cross and in control flowers of *D. Stramonium* even though this process was closely observed in the hope of detecting differences between them. Fertilization in the cross between *D. Stramonium* and *D. Metel* takes place and the fact that fusion occurs cannot be doubted.

It should be added that the second synergid in the embryo sac remains visible until the end of the fertilization process (fig. 11) and then disintegrates. The pollen tube after releasing the male gametes, always contains two so-called x-bodies (fig. 11). They are deeply stained by the Feulgen reaction and must therefore contain chromatin. They are evidently disintegrating nuclei; one belongs to the synergid through which the pollen tube grew, and the other is the tube nucleus of the pollen tube.

### III. THE PROEMBRYO AND ENDOSPERM DEVELOPMENT

After fertilization is complete in the cross *D. Stramonium*×*D. Metel* the zygote and endosperm nuclei divide, but the amount of time that elapses between fertilization and cell division is variable. Several hours always pass by before the latter occurs. It is the triploid endosperm nucleus which starts to divide. Various stages of its division were found in capsules taken from cross pollinated flowers 68–72 hours after pollination. In other ovules division of the endosperm nucleus did not begin until four days after pollination. In exceptional cases the division started on the fifth day.

Figures at a magnification of 1800 diameters.

Fig. 15—*D. Str.*×*D. Metel:* 36 chromosomes in metaphase plate of an endosperm cell, 5 days after pollination.

Fig. 17—*Same:* 24 chromosomes in metaphase plate of a zygotic nucleus.

Fig. 23—*D. Str.*×*D. Str.:* 36 chromosomes in metaphase plate of an endosperm cell, 7 days after pollination.

Figure 14 is a drawing of a section through one of the ovules fixed three days after pollination. It shows a pollen tube which has grown through the micropyle, an undivided zygote and two endosperm cells. The presence of these two separate endosperm cells is in agreement with Guignard's (1902) observations that the endosperm in *D. laevis* (our white inermis stramonium) has no stage of free nuclear division. The second division takes place soon afterwards but does not always occur simultaneously in the two daughter nuclei. Other divisions follow and soon a number of normal appearing cells are formed.

Each cell contains a rich amount of cytoplasm and a large nucleus. Especial attention was devoted to the division process in these endosperm cells; it seemed to be normal. Thirty-six chromosomes were counted in numerous endosperm cells at metaphase stage. It is thus seen that the endosperm cells were 3n, each of them having one paternal and two maternal chromosome sets (fig. 15).

Normal appearing endosperm cells without any visible trace of dis-

integration are to be found in young capsules fixed from three to six days after pollination. They predominate also at the seventh day after pollination and in some seeds at the eleventh or thirteenth day. However, disintegration sometimes occurs earlier—at the sixth day after pollination. Figure 20 shows such a case; the disintegration only begins in the proembryo but the endosperm cells are already dead. The epithelial cells which surround the embryo sac are of enormous size in comparison with those of normal seeds or even with those of hybrid seeds in which the disintegration of the endosperm has been delayed (fig. 21). In the majority of seeds fixed on the eighth, ninth to fourteenth day after pollination all endosperm cells are usually dead and their resorption proceeds rapidly.

Division of the zygotic nucleus begins usually on the fifth, sometimes on the fourth day after pollination, that is, one or two days after the endosperm nucleus has completed four to five divisions. Figure 16 shows such a case. It is a section from an ovule in which 18 endosperm cells are already formed and the zygotic nucleus goes through its first division. It contains 24 chromosomes in metaphase (fig. 17). Figure 18 shows a young proembryo with two cells, figure 19 shows one with four cells. They were found among a large number of other proembryos in a capsule fixed five days after pollination. In capsules six to seven days old, proembryos with two to four cells are common while those with five and six cells are rare. A proembryo with as many as eight cells was found only once. It is evident that, though fertilization can take place and two or at most three successive divisions in the young sporophyte can occur, something prevents further growth.

Disintegration proceeds independently in the proembryo and in the endosperm cells. Frequently the proembryo has been resorbed before the endosperm begins to disintegrate and a cavity approximately equal to its size and shape results (fig. 21). In other cases the proembryo is still present while the endosperm is partly resorbed (fig. 20).

The early stages of development of the endosperm in *D. laevis* have been given by Guignard (1902), and a detailed study of the proembryo development in *D. Stramonium*, *D. laevis* and *D. Tatula* (which may be included in *D. Stramonium*) was made by Souèges (1922). No detailed description of proembryo nor of endosperm development in controls need be given in the present paper, since the results confirm the findings of these authors. Only few details, such as chromosome counts in the endosperm cells, may be added. Figure 22 shows the division of an endosperm nucleus and an undivided zygote, figure 23 has a metaphase plate from a triploid endosperm cell in which 36 chromosomes can be counted. The first drawing was made from a capsule four days old, the second from a capsule seven

days old. A three-celled proembryo in which the lower cell is dividing is shown in figure 24, and a four-celled proembryo is shown in figure 25. Both proembryos were found in a capsule five days old. If we compare the development of the endosperm and proembryo in the cross *D. Stramonium* ×*D. Metel* with *intra se* crosses of *D. Stramonium* it will be seen that no visible differences are detectable at early stages. The division of the zygotic nucleus occurs in both at the same relative time. The endosperm nucleus divides first, then after about four or five divisions have taken place the zygotic nucleus starts dividing. The zygotic nucleus contains 24, the endosperm nucleus 36 chromosomes.

Although the early stages of growth appear to be indistinguishable, a difference is noticeable in the rate of further development. Crosses of *D. Stramonium* with *D. Metel* develop slower than those of the control. It is clearly seen six to seven days after pollination. At this time the proembryo of the control consists of 16 to 30 cells while the proembryo of the cross has only four to six cells. The same difference in rate of development was observed in the two kinds of endosperm. That of the control showed about 70–120 cells in transverse section while comparable figures for the cross are 18–30 cells. This latter number corresponds to a total of 80 to 90 cells for the endosperm of the cross. This difference in number of cells increases rapidly in later stages because of the continued division of the proembryo and endosperm in the control, while in the cross there is a gradual arrest of development. In later stages only isolated dividing cells may be found in the disintegrating endosperm of the cross. The division of the proembryo stops earlier. By the end of the second week, except in the few cases mentioned above, large cavities, due to resorption, are found in place of the seed proper. It is of interest that the seed coat develops normally and does not seem to be affected by the disintegration of the seed.

Michaelis (1925) reported the embryological history in crosses between *Epilobium* species, but did not study their chromosomes. In certain combinations the hybrid proembryo developed normally but the seed failed to germinate. In others a number of irregularities accompanied the development of the proembryo (no cell wall formation in the proembryo after division, giant cells in the endosperm, etc.). In a certain combination the proembryo degenerated at early stages after a few cells had been formed. This latter type resembles the data reported in this paper.

As has been shown, failure to form viable seeds in the cross *D. Stramonium*×*D. Metel* is not due to irregularities of pollen-tube growth in the foreign style nor to lack of fertilization. Both female nuclei fuse with male gametes, forming a 2n zygote and a 3n endosperm. In these cells the occurrence of the first division after fertilization is not surprising since the

chromosomes are already split in anticipation of this division. But the fact that chromosomes of *D. Metel* participate in further divisions indicates that they can grow and divide in the *D. Stramonium* cytoplasm, at least for a limited number of divisions.

The later stages observed in both proembryo and endosperm of these hybrid seeds give the impression that disintegration of the cells prevents further divisions of the chromosomes rather than that failure of chromosomes to divide is a primary factor preventing further development. The present paper is one of a series on the species problem in the genus *Datura*. It gives information regarding the stages in an incompatible cross at which development is arrested but leaves to later investigations the mechanisms by which this block to hybridization is accomplished.

SUMMARY

1. In mature microspores of *D. Metel* and *D. Stramonium* there is a smaller generative nucleus and a larger tube nucleus.

2. In the cross *D. Stramonium*×*D. Metel* two male gametes are formed by division of the generative nucleus in pollen tube.

3. One male gamete fuses with the egg nucleus, the other with the central nucleus, as in the parent species.

4. The zygote is 2n and the endosperm 3n, as in control (*D. Stramonium*).

5. Endosperm begins to divide before the zygote.

6. Cells of the endosperm appear normal up to about the seventh day, when disintegration begins.

7. Proembryos up to about the fifth or seventh day appear normal but do not form more than eight cells after which disintegration and resorption occurs.

8. Disintegration may occur first in either the proembryo or endosperm.

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## Explanation of figures 1–13

Figures, except when noted, at a magnification of 900 diameters.

c—central nucleus; g—generative nucleus; p—pollen tube; s—synergid; t—tube nucleus; x—X-body; ♂—male gamete; ♀—egg nucleus

Fig. 1—*D. Stramonium:* 12 chromosomes, metaphase in microspore.

Fig. 2—*D. Metel:* generative and tube nuclei in microspore.

Fig. 3—*Same:* germination of microspore: generative and tube nuclei. Microspore fixed 1½ hour after having been placed on agar.

Fig. 4—*Same:* generative nucleus at prophase stage in pollen tube, 7 hours after pollination. ×1050.

Fig. 5—*D. Str.:* generative nucleus at metaphase stage in pollen tube showing 12 chromosomes, 9 hours after pollination.

Fig. 6—*Same:* 2 male gametes and tube nucleus in pollen tube, 9 hours after pollination.

Fig. 7—*D. Str.*×*D. Str.:* part of an embryo sac before fertilization, pollen tube in micropyle, 2 male gametes near the tip of the pollen tube; tube nucleus behind; 2 synergids, egg cell and central nucleus; 29 hours after pollination.

Fig. 8—*D. Str.*×*D. Metel:* two male gametes and tube nucleus in pollen tube, 23 hours after pollination.

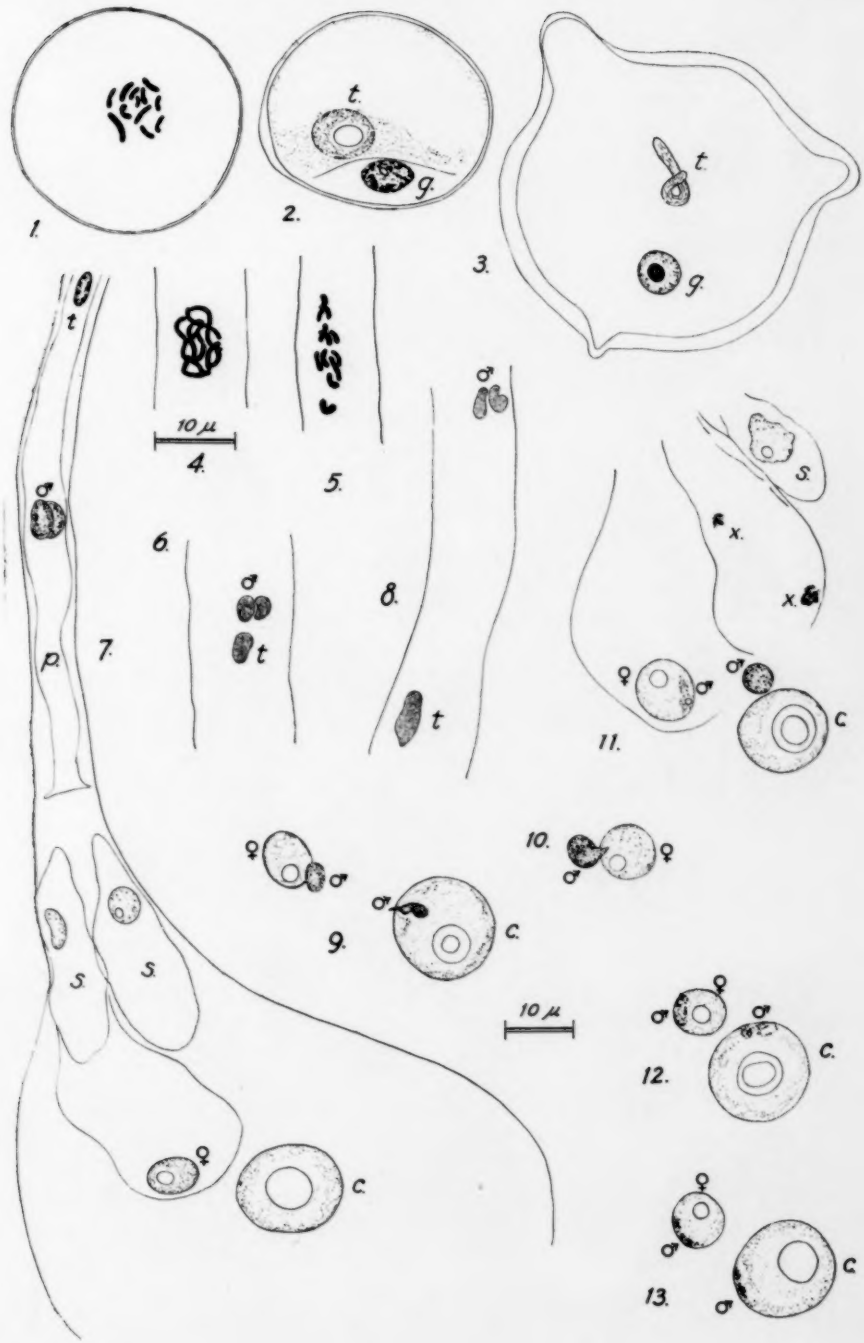
Fig. 9—*Same:* double fertilization, male gametes in contact with egg and central nuclei, 68 hours after pollination.

Fig. 10—*D. Str.*×*D. Str.:* male gamete in contact with egg nucleus, 29 hours after pollination.

Fig. 11—*Same:* double fertilization, fusion almost completed in egg nucleus; male gamete approaches the central nucleus; two *X* bodies in pollen tube, 1 synergid; 29 hours after pollination.

Fig. 12—*D. Str.*×*D. Metel:* double fertilization; fusion almost completed, 68 hours after pollination.

Fig. 13—*D. Str.*×*D. Str.:* double fertilization, fusion almost completed, 29 hours after pollination.




SATINA AND BLAKESLEE: FERTILIZATION IN DATURA

UNIV. OF MICH.

**Explanation of figures 14, 16, 18–22, 24 and 25**

Figures, except when noted, at a magnification of 900 diameters.

e—endosperm; p—pollen tube; z—zygote

Fig. 14—*D. Str.* × *D. Metel:* 2 endosperm cells, undivided zygote, pollen tube, 3 days after pollination. ×200.

Fig. 16—*Same:* division of zygotic nucleus, two endosperm cells; lower cell at anaphase, 5 days after pollination.

Fig. 18—*Same:* 2 celled proembryo, 5 days after pollination.

Fig. 19—*Same:* 4 celled proembryo, 5 days after pollination.

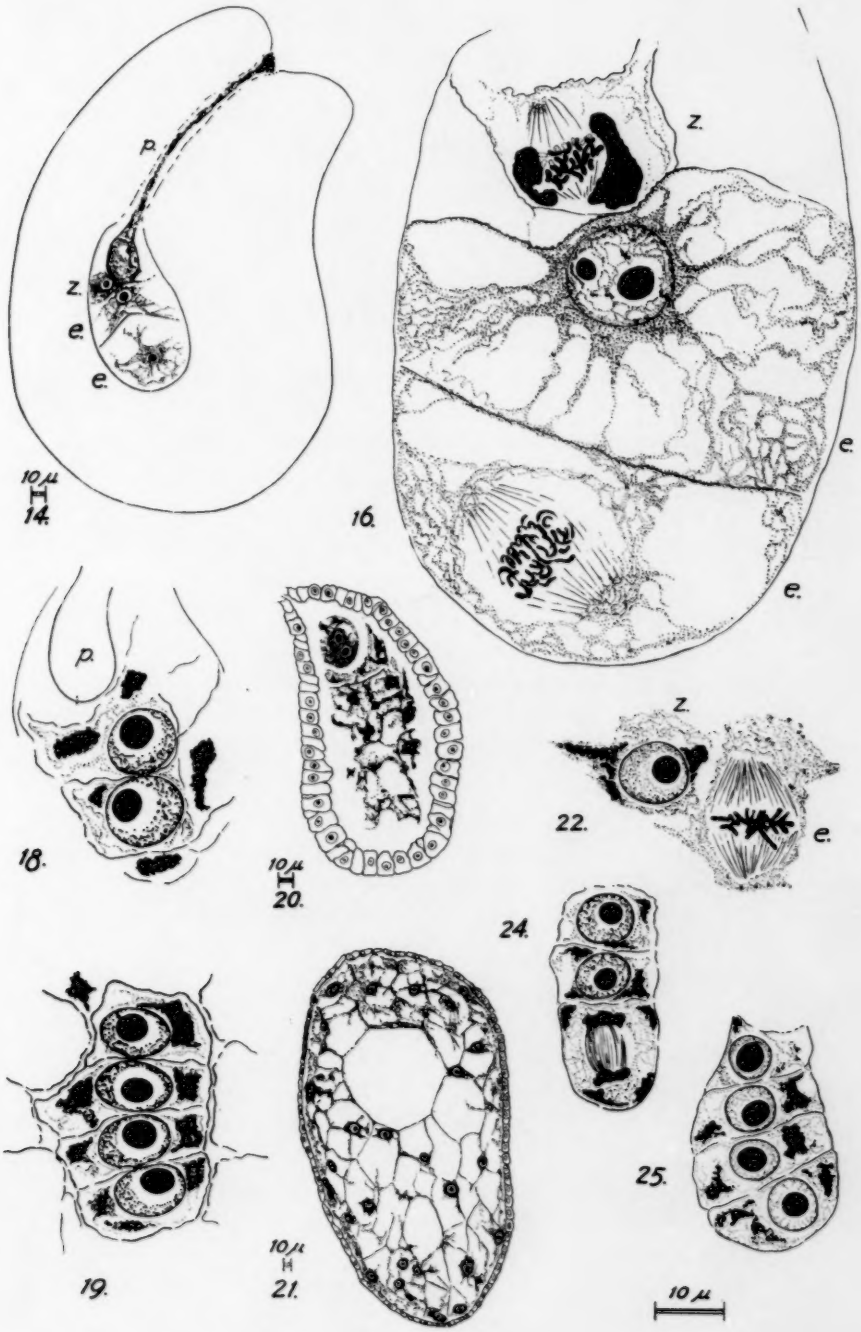
Fig. 20—*Same:* advanced stage of disintegration in endosperm and early stage of disintegration in proembryo; enlarged cells of the epithelium around the embryo sac, 6 days after pollination. ×200.

Fig. 21—*Same:* normal endosperm with proembryo entirely resorbed; 11 days after pollination. ×100.

Fig. 22—*D. Str.* × *D. Str.:* 1st endosperm division showing only part of the chromosomes; zygote undivided, 4 days after pollination.

Fig. 24—*Same:* 3 celled proembryo, 5 days after pollination.

Fig. 25—*Same:* 4 celled proembryo, 5 days after pollination.




SATINA AND BLAKESLEE: FERTILIZATION IN DATURA

# Differential distribution of a phytohormone in the developing leaf of Nicotiana, and its relation to polarized growth

GEORGE S. AVERY, JR.

(WITH FIVE TEXT FIGURES)

## INTRODUCTION

A rapidly increasing interest in plant growth hormones (variously referred to as Wuchsstoff, growth substance, growth regulator and auxin) has been aroused since Went (1928) showed that quantitative determination was possible by the use of *Avena* coleoptiles. Supplementary (Thimann 1934, Went, 1934) or other methods suggested more recently have extended the range of possibilities.

The phytohormones so far reported seem closely linked with growth by cell enlargement, although Almoslechner (1934) reports distinctly different hormones for cell enlargement (Wuchsstoff A) and cell division (Wuchsstoff B) in yeast. Kögl has recently (1935) given evidence that minute amounts of a hormone which he designates as "Biotin *a*" may accelerate cell division in yeast as much as 1400%, a figure many times greater than that given by Almoslechner for Wuchsstoff B.

The generic term *auxin* for phytohormones which bring about cell enlargement has been proposed by Kögl. The advantage of this uniform terminology is clear from the following. Auxin *a* and *b* have been isolated from a number of plant materials (Kögl, Erxleben and Haagen-Smit, 1934*a*) and crystallized in pure form. Auxin *a* has the chemical formula $C_{18}H_{32}O_5$, and auxin $b = C_{18}H_{30}O_4$. Their structural formulae have been reported recently (Kögl and Erxleben, 1934). A substance identical with $\beta$-indolyl-acetic acid ($C_{10}H_9O_2N$) has been isolated from urine and from yeast and the name *heteroauxin* has been suggested for it (Kögl et al, 1934*b*, 1934*c*).

Most investigations on growth effects of auxin have been concerned with *Avena* seedlings, although a wide variety of materials have been subjected to examination in the past two years. The presence of auxin in leaves has been demonstrated in numerous instances for *Avena* (the coleoptile is interpreted as the second, or sheathing leaf of the plant, Avery, 1930), *Vicia faba* (Thimann and Skoog, 1934), and *Ipomoea* (Koning, 1933) But there has been no work on its distribution in relation to growth of foliage leaves. The purpose of this study was, first, to determine the concentration of auxin in leaves of varying ages: second, to determine whether differential distribution of auxin within the leaf might be correlated with differential growth intensity, or whether all parts of the leaf have it in

equal concentrations; third, to find whether it accumulates in certain portions of the leaf, or is transported in certain tissues, i.e., vascular tissue, etc. (next to nothing is known of its path of transport in mature plants); fourth, to determine whether its movement is polar, as has been demon-

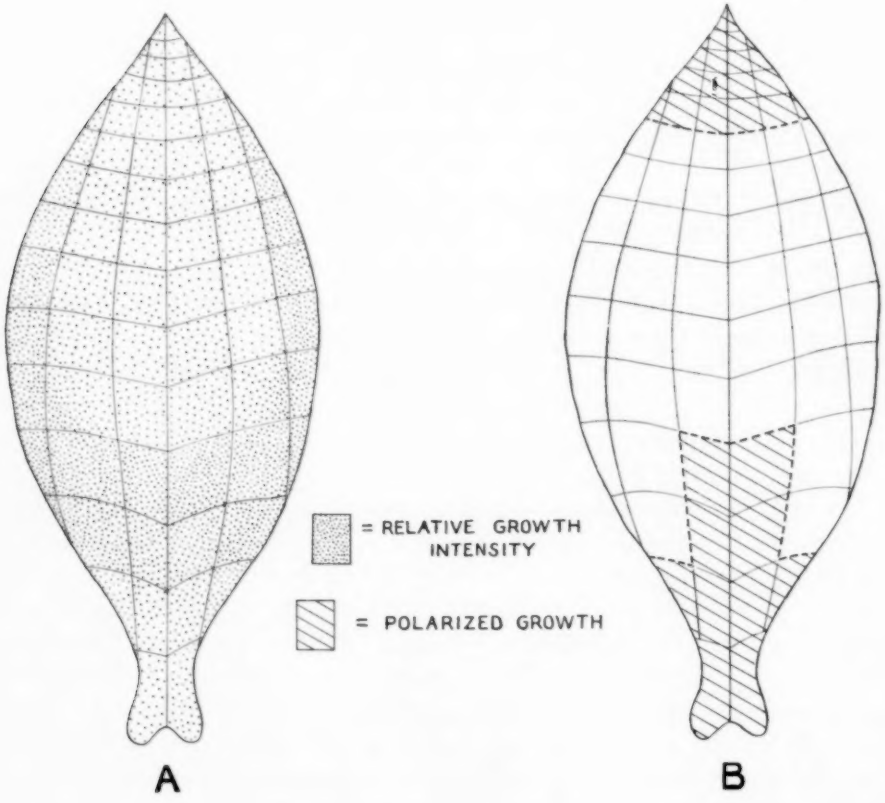



Fig. 1. After Avery, 1933. A, the density of the dots indicates relative growth intensity (*localized growth*) in the different portions of a developing leaf. The marginal and basal regions undergo the greatest growth. B, the segments indicated in the distal and proximal portions of the leaf show a relatively greater increase in length than in width. While this *polarized growth* is not so pronounced at the apex, it is very striking at the basal end of the leaf (where it is correlated with higher concentrations of auxin).

strated in *Avena* coleoptiles; fifth whether it is actually a limiting factor in leaf growth, and sixth, if a limiting factor, whether it is influencing cell division, cell enlargement, or both.

Leaves of *Nicotiana* were chosen for study because of their large size, and because their growth has already been investigated. It has been shown

(Avery, 1933) that relative growth rates differ in different portions of the leaf, and that growth intensity (*localized growth*) is greatest near the proximal end and at the margins (fig. 1 A). It has been shown also that the distal and proximal portions of the leaf, more particularly the latter, show a relatively greater increase in length than in width (fig. 1 B, *polarized growth*). Is auxin in part responsible for either localized or polarized growth?

## MATERIALS AND METHODS

Two species and several varieties of *Nicotiana* were used in these experiments: *N. sylvestris* Speg. & Comes, pedigree number 34100; $F_1$ plants of *N. Tabacum*×tetraploid *N. sylvestris* no. 34334; *N. Tabacum* L., varieties *purpurea* (no. 34042), Connecticut Broadleaf (no. 34043) and R. E. Clausen's Broadleaf (no. 34045). Most were supplied through the kindness of Drs. Thomas H. Goodspeed and Helen Mar Wheeler of the University of California.

Young plants were removed from the field near Berkeley on July 2, and were replanted in 8 inch pots in the greenhouse in Pasadena on July 5, 1934. There were from five to eight good sized leaves on each plant at the time of transplanting, and three to four weeks later most of the plants had from ten to eighteen leaves.

The main procedure followed for the extraction of auxin was the diffusion method of F. W. Went (1928). Definite portions were cut from the leaf and rolled into compact cylinders which were tied with fine copper wire (fig. 2 A, B, C). The proximal cut end was touched with 10% gelatin and the cylinder then placed on the standard agar block (Dolk, 1930). Diffusion was allowed to continue for the usual two hour period in a moist chamber. Where the diffusion method was used on whole leaves of a length greater than six or eight centimeters, the leaves were hung from a rack in a large moist chamber, and the agar block was attached to the cut petiolar end of the suspended leaf.

The chloroform method of Thimann (1934) was used as a check on the diffusion method, and in other instances where it seemed desirable to extract all the auxin present at any given time. It consists, in brief, of immersing the fresh material in chloroform, adding a small amount of hydrochloric acid, and grinding thoroughly. The chloroform layer containing the auxin is separated from the residue, and is then evaporated off. The lipoidal material remaining is taken up in a very small volume of water to which is added an equal volume of 3% agar. The standard size block is then cast from this.

Quantitative determinations of the extracted auxin (from both the

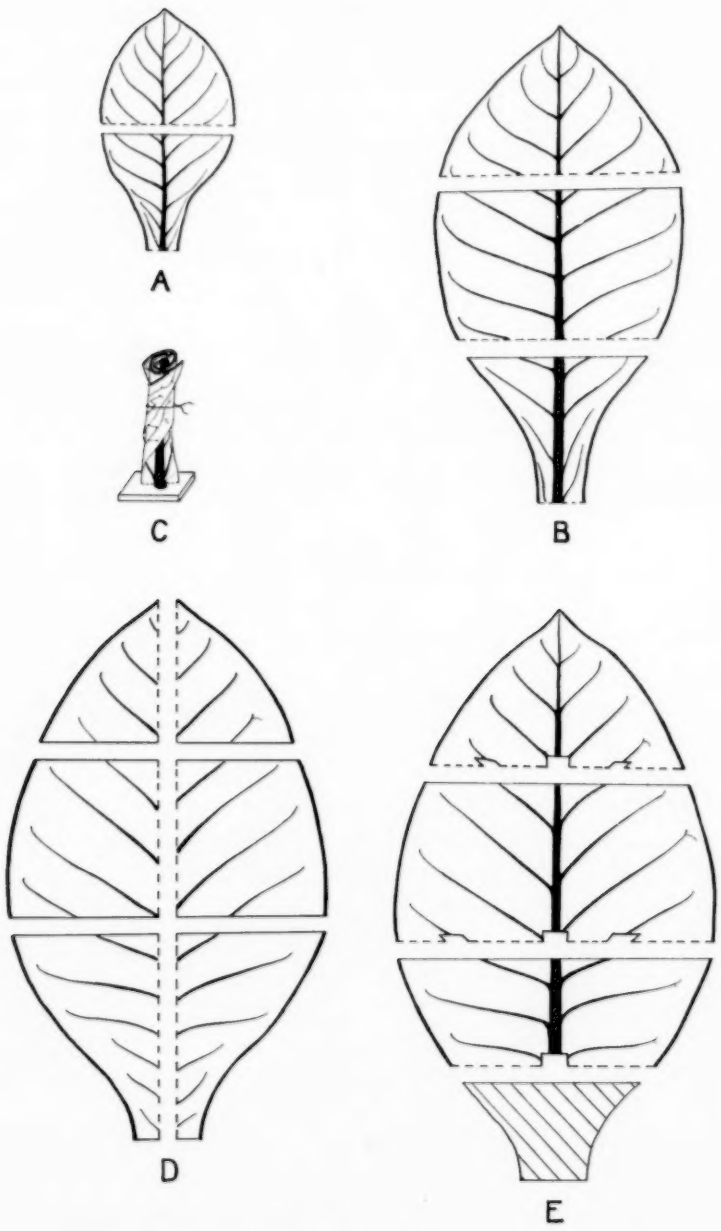



Fig. 2. Diagrams showing how young (A) and older (B) leaves were cut into segments, and how the auxin was "diffused" into the agar block (C); D shows how the midrib was removed so that it and the remainder of the leaf might be tested separately; E shows how the midrib and large lateral veins were cut back in one experiment, so as not to come in contact with the agar. The portion of the leaf placed in contact with the agar block is indicated by a discontinuous line (- - -).

diffusion and chloroform methods) were made according to the Went (1928) technique. The test depends upon the curvature produced in decapitated *Avena* coleoptiles when small segments of the above mentioned agar blocks containing auxin are applied to one side of the long stump remaining after decapitation. It has been shown (Thimann and Bonner, 1932, and van der Wey, 1932) that the result depends on relative ***concentration*** rather than upon the ***amount*** of the auxin present in the blocks. All tests were carried out in a dark room especially designed to maintain temperature at 25° to 27° C., and relative humidity at approximately 90%.

Results are given in "plant units" (Dolk and Thimann, 1932) where 1 *p. u.* equals 1° curvature of the coleoptile stump, brought about in 110 minutes by the presence of the auxin contained in the agar block. Such a plant unit is equal to only four-tenths of the *Avena* unit ("AE") of Kögl, Haagen-Smit and Erxleben (1933).

In certain experiments heteroauxin, synthesized by Dr. K. V. Thimann, was thoroughly mixed with lanolin (90 mg. to 10 gms.) and applied externally to leaves, similar to the lanolin method of Laibach (1933) in which orchid pollen was used as a source of the auxin.

## CONCENTRATION OF THE AUXIN IN YOUNG AND OLDER LEAVES

The terminal inflorescences were just appearing at the tips of the plants as the leaves of each variety were tested. The leaves immediately below were still very young, while those from successively lower levels were correspondingly older (table 1). Each figure represents the average of two leaves, and the number of plant units are given both for the leaf as a whole, and on a per square centimeter basis.

It is clear that the very young leaves, notably the first two below the embryonic inflorescence, are high in growth substance. There were only nine leaves on the plants tested July 13, and the upper five of these were still undergoing appreciable growth. The lower four were increasing in length only slightly, and were therefore interpreted as being nearly mature. This greater degree of maturity is correlated with a rapid dropping off in the concentration of the auxin. Corresponding results were obtained from tests on leaves of another variety nearly two weeks later. In this case there were more leaves on the plants, and the lower leaves were mature. Complete maturity is correlated with a total absence of auxin.

It seems reasonable to conclude from these tests that auxin is present only in growing leaves and that its concentration is roughly inversely proportional to the age of the leaf.

TABLE 1

*Auxin concentration in the inflorescence, and in leaves of various ages from different levels on the stalk. Concentrations are given in plant units ("p.u.")*[1]

| LEAF NO. BELOW INFLORESCENCE | N. TABACUM VAR. PURPUREA, JULY 13 | | | N. TABACUM VAR. CONNECTICUT BROADLEAF, JULY 25 | | |
|---|---|---|---|---|---|---|
| | AVERAGE AREA PER LEAF IN SQ. CM. | TOTAL P.U. | AVERAGE P.U. PER SQUARE CM. OF LEAF | AVERAGE AREA PER LEAF IN SQ. CM. | TOTAL P.U. | AVERAGE P.U. PER SQUARE CM. OF LEAF |
| Inflorescence | | 32 | | | 53.4 | |
| 1 | 5.00 | 46.5 | 9.37 | 1.32 | 45.9 | 34.8 |
| 2 | 6.61 | 55 | 8.32 | 5.09 | 56.5 | 11.10 |
| 3 | 17.8 | 58.5 | 3.28 | 20.19 | 60 | 2.97 |
| 4 | 38.12 | 40 | 1.04 | 27.54 | 54 | 1.95 |
| 5 | 31.2 | 37.5 | 1.20 | 55.35 | 39.5 | 0.713 |
| 6 | 44.4 | 24 | 0.54 | 109.77 | 38.5 | 0.350 |
| 7 | 61.0 | 26 | 0.425 | 120.93 | 29.5 | 0.243 |
| 8 | 86.93 | 9.9 | 0.115 | | 11 | |
| 9 | 140.67 | 18 | 0.127 | 247.83 | 11.4 | 0.045 |
| 10 | | | | 301.03 | 11.5 | 0.038 |
| 11 | | | | 277.80 | 0.0 | |
| 12 | | | | 187.12 | 0.0 | |
| 13 | | | | 139.61 | 0.0 | |
| 14 | | | | 158.51 | 0.0 | |
| 15 | | | | 164.48 | 0.0 | |

[1] One plant unit equals 1° curvature of the coleoptile stump, brought about in 110 minutes by the presence of the auxin contained in the agar block.

## DIFFERENTIAL DISTRIBUTION OF AUXIN IN THE LAMINA OF YOUNG AND MORE NEARLY MATURE LEAVES

### *The concentration gradient from tip to base of leaf*

Leaves at various stages of development, from very young to nearly mature, were divided into halves, thirds, or quarters and tested by the

diffusion method (fig. 2). Auxin is present in all portions of the leaf in young rapidly growing leaves such as A–C, F and G (table 2 and fig. 3),

TABLE 2

*Distribution of auxin in different portions of the leaf in leaves of varying ages.*

| N. TABACUM VAR. PURPUREA | DATE | AREA IN SQ. CM. | TOTAL P.U. | P.U. PER SQ. CM. |
|---|---|---|---|---|
| A. Leaf 4.5 cm. long[2] | | | | |
| terminal half | July 13 | 3.45 | 24.9 | 7.21 |
| basal half | | 3.67 | 42 | 11.44 |
| B. Leaf 8 cm. long[2] | | | | |
| terminal half | July 13 | 13.70 | 27 | 1.97 |
| basal half | | 14.61 | 45.9 | 3.14 |
| C. Leaf 10 cm. long[2] | | | | |
| terminal third | July 13 | 6.58 | 20.4 | 3.10 |
| middle third | | 15.48 | 36.9 | 2.38 |
| basal third | | 9.77 | 72 | 7.26 |
| D. Leaf 18 cm. long (practically mature) | | | | |
| terminal quarter | July 6 | | 0.0 | |
| upper middle quarter | | | 0.0 | |
| lower middle quarter | | 32.68 | 20.4 | 0.63 |
| basal quarter | | 15.03 | 33.6 | 2.23 |
| N. TABACUM×N. SYLVESTRIS | | | | |
| E. Growing point,[2,3] (with accompanying leaves up to 5 cm. long) | July 22, 23 | | 112.8 | |
| F. Leaf 9 cm. long[3] | | | | |
| terminal quarter | July 18 | 5.80 | 24 | 4.13 |
| upper middle quarter | | 11.35 | 82.2 | 7.24 |
| lower middle quarter | | 10.12 | 96 | 9.48 |
| basal quarter | | 4.9 | 130.2 | 26.57 |
| G. Leaf 13 cm. long[3] | July 18 | | | |
| terminal quarter | | 10.90 | 21 | 1.92 |
| upper middle quarter | | 17.93 | 72 | 4.01 |
| lower middle quarter | | 19.74 | 78 | 3.95 |
| basal quarter | | 9.54 | 141 | 14.77 |

[2] Data from average of 2 leaves.

[3] There was a total absence of auxin in growing points and leaves of plants kept in darkness 5 days before testing. All such duplicate tests were on material of size and parentage comparable to E–G.

but is unequally distributed. There is a distinct gradient from the tip to the base of the leaf, the concentration being much higher near the base. In a nearly mature leaf, such as D, the auxin has disappeared completely from the distal half, while the proximal portion still has a low concentration present.

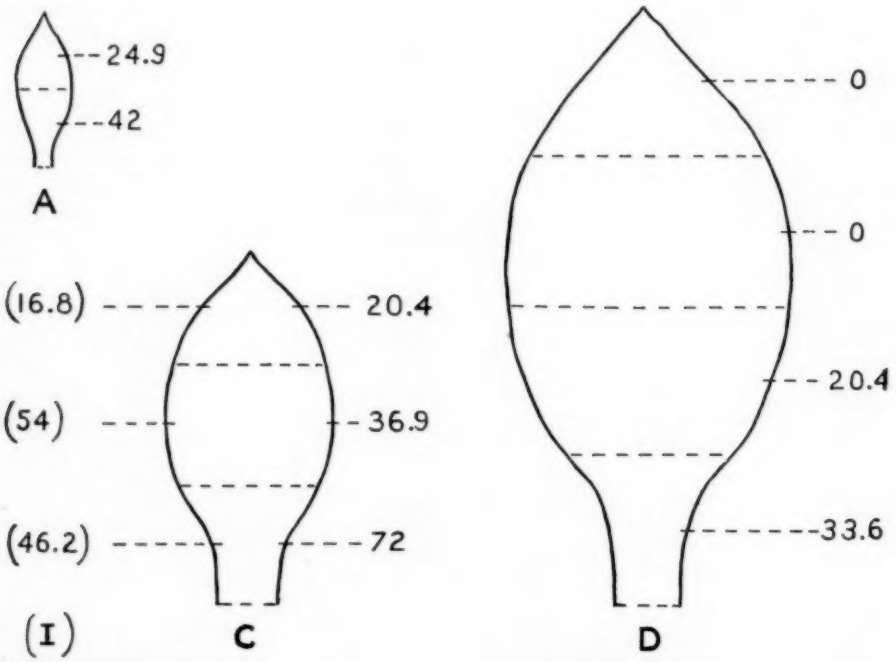



Fig. 3. Diagrams of young (A) and older (C and D) leaves, showing relative concentrations of the auxin in different portions. All figures are in plant units (*p. u.*) and the leaves diagrammed are those similarly lettered in table 2. The data in parentheses are taken from leaf I, table 3; this leaf had been in darkness for ten days, followed by one day in the greenhouse. The auxin concentration gradient shown in A and C is dependent upon accumulation of the auxin in the midrib and its movement toward the base of the leaf (in contrast with leaf I, in which accumulation has not had time to take place). Note its eventual disappearance at the distal end (D) as the leaf grows older.

The chloroform extraction method was used to check the above results. On July 21, three leaves were removed from near the middle of the stalk of a plant of *N. sylvestris*. The leaves averaged 26 centimeters long, and were cut into quarters, rather than into thirds as shown in figure 2, B. The yield in plant units was as follows:

terminal and upper middle quarter..... (average)..... { 0.17 *p. u.* per sq. cm., or 5.75 *p. u.* per gram green wt.

lower middle quarter . . . . . not tested

basal quarter . . . . . { 0.8 *p. u.* per sq. cm., or / 9.43 *p. u.* per gram green wt.

Thus the concentration of the auxin as shown by the chloroform method corresponds with that of the diffusion method recorded for leaves A–G in table 2.

The fact that there is a definite concentration gradient from tip to base of leaf, and that the auxin tends to disappear distally as the leaf approaches maturity, might be interpreted in two possible ways: either there is a greater amount formed in the proximal portion of the leaf, or, it is formed approximately equally throughout the lamina, but is accumulated in the proximal portion.

### *Accumulation and transport*

Scattered bits of evidence pointing to accumulation and transport in veins were verified by the following tests. On July 26, the midrib and main lateral veins of a large but still growing leaf of the variety Connecticut Broadleaf (28 cm. long) were cut away from the remainder of the lamina and tested separately by the chloroform method:

| | WEIGHT | TOTAL YIELD OF AUXIN | AUXIN YIELD PER GRAM GREEN WEIGHT |
|---|---|---|---|
| Midrib and main lateral veins | 2.4 grams | 144 p.u. | 60 p.u. |
| Intervenous portions of leaf | 2.1 grams | 57 p.u. | 27.14 p.u. |

The intervenous portions yielded less than half the auxin shown to be present in the veins.

In another experiment (diffusion method) on July 27, two leaves of *N. sylvestris* were used, each being about 18 cm. in length. The lateral veins were cut from the intervenous portions and midribs, and tested separately: 11 lateral veins taken from the distal half of the leaf showed no appreciable concentration of the auxin; 12 lateral veins taken from the proximal half of the leaf gave 19.8 *p. u.* (both leaves); intervenous material from these same leaves showed no appreciable concentration. The midribs from the same two leaves were divided into three equal lengths and the proximal 3 cm. of each of these lengths was tested: distal 3 cm., negligible; middle 3 cm., 37.2 *p. u.* (both leaves); proximal 3 cm., 43.2 *p. u.* (both leaves).

It will be shown that auxin occurs in approximately equal concentrations in the intervenous regions whether it be at the tip or the base of

growing leaves. This evidence, together with the above data, indicates that accumulation and transport take place largely in the midrib and main lateral veins.

*Movement of the hormone is polar*

On July 17 two middle sized rapidly growing leaves were cut into thirds, and the *distal* end of each third was applied to the agar block (the reverse of the usual method of placing the proximal end on the block for diffusion). The results were negative! That the movement of the auxin is strictly a polar phenomenon has been reported in several instances by other investigators.

In the leaf shown in figure 2, D, the hormone moved inward from the margin of the leaf toward the midrib. In a similarly prepared leaf, the margin was also cut off and the outer cut edge placed in contact with the agar for diffusion. No yield was obtained. From such results it is clear that the direction of transport is from tip toward base of leaf, and from lateral veins in toward the midrib.

PRODUCTION OF THE AUXIN IN YOUNG GROWING LEAVES

The material for this series of experiments consisted of $F_1$ plants of *N. Tabacum*×*N. sylvestris*. Foregoing data suggested that the auxin probably was being produced throughout the leaf, even though accumulation and transport were taking place largely in the veins.

The experimental evidence shows (see footnote 3, table 2) that when plants are kept in darkness for a few days before testing, the auxin disappears from the growing point and leaves. The growing point recovers its usual concentration of auxin after a day in the greenhouse, as shown in table 3.

Leaves show a similar recovery but do not have time to accumulate an appreciable amount of the hormone in their proximal portion. In leaves I, J, and K which received this treatment, the central region of the leaf appears to be producing a greater amount of the auxin than corresponding portions of control leaves L and M. If computed on the basis of concentration per square centimeter of lamina, the results are roughly of the same order in both the experiment and the controls. It is clear from this that neither total concentration nor calculations based on area necessarily give a true picture of the results. The explanation for this lies in the differing proportions of midrib and intervenous tissues in different parts of the leaf; for example, the proportion of intervenous material to veins is quite different at the tip, middle, and base of the leaf (fig. 2, B). If computed on the basis of green weight, the direction of the concentration gradient is

TABLE 3

*Distribution of the auxin in different portions of leaves. Plants were kept in darkness ten days, then in the greenhouse one day before testing. Leaves L and M were controls. All data for "lamina" include the midrib.*

| N. TABACUM×SYLVESTRIS F1 JULY 25 (PLANTS PLACED IN DARKROOM FOR 10 DAYS, THEN 1 DAY IN GREENHOUSE) | AREA OF LAMINA IN SQ. CM. | WEIGHT IN GRAMS | TOTAL P.U. | P.U. PER SQ. CM. OF LAMINA | P.U. PER GRAM GREEN WEIGHT |
|---|---|---|---|---|---|
| Growing point, with accompanying leaves up to 4.5 cm. long | | | 109.8 | | |
| I. Leaf 10 cm. long growing rapidly: | | | | | |
| terminal third of leaf | 8.5 | 0.15 | 16.8 | 1.97 | 112 |
| middle third | 14.9 | 0.55 | 54 | 3.62 | 98.2 |
| basal third | 7.6 | 0.45 | 46.2 | 6.07 | 102.7 |
| J. Leaf 17.5 cm. long still growing, but not rapidly: | | | | | |
| terminal quarter of leaf | 14.19 | 0.26 | 22.2 | 1.56 | 85.4 |
| upper middle quarter | 31.93 | 1.5 | 66 | 2.06 | 73.5 |
| lower middle quarter | 20.51 | 1.5 | 54 | 2.63 | 73.5 |
| lower quarter | 7.87 | 0.74 | 34.2 | 4.34 | 46.2 |
| K. Leaf 18 cm. long, still growing: | | | | | |
| terminal third | 29.7 | 0.55 | 22.8 | 0.76 | 41.4 |
| middle third | 44.2 | 1.3 | 51.6 | 1.16 | 39.7 |
| basal third | 12.7 | 1.2 | 32.4 | 2.55 | 27 |
| (plants grown in greenhouse, same hybrid, July 25) | | | | | |
| L. Leaf 16 cm. long, still growing: | | | | | |
| terminal third | 27.5 | 0.5 | 0.0 | 0.0 | 0.0 |
| middle third | 38.3 | 1.25 | 43.8 | 1.4 | 35 |
| basal third | 15.6 | 1.1 | 70.8 | 4.5 | 64 |
| M. Two leaves, one 16, the other 18 cm. in length: | | | | | |
| segment from terminal portion—*average per leaf* | 14.4 | .425 | 27.5 | 1.9 | 64.7 |
| strip across middle portion of leaf | 21.15 | .435 | 47.5 | 1.97 | 100.0 |
| strip across leaf about 3 cm. above base | 12 | .375 | 52.8 | 4.4 | 139.1 |

reversed in leaves I, J, and K of leaves which were taken from dark treated plants (table 3). With the midrib adding considerable weight to the basal portion of the leaf and the leaf having had little opportunity to accumulate the auxin, it might well be expected that the concentration per gram of green weight would be proportionally lower in this region. Control leaves L and M, on the other hand, show the same sort of gradient as leaves A–G (table 2).

In order to further test whether the auxin is produced in approximately equal concentrations throughout the lamina, a leaf 12 cm. in length (*N. Tabacum*×*N. sylvestris*) was divided into thirds on July 24. Its midrib and large lateral veins were cut back so they would not be in contact with the agar at the time of diffusion (fig. 2, E). Any diffusion of the auxin which might take place should therefore come from the so-called intervenous regions, where only small veins are present. Diffusion was allowed to go on for four hours, or double the usual length of time. The yield for the distal third was 27 *p. u.*, the middle third 31 *p. u.*, and the proximal third 31 *p. u.* Another leaf of the same parentage, 13 cm. in length, was cut into thirds on July 24. The midrib was entirely removed (fig. 2, D) and the cut edge nearest the midrib was placed downward on the agar. The main lateral veins were left intact. After four hours diffusion the segments from the middle of the leaf gave 40 *p. u.*, those from the proximal end, 41. The terminal segments did not give satisfactory tests.

The results from this series of preliminary experiments show that the hormone is *produced* in approximately equal amounts over the whole lamina, a fact that is usually masked by the *accumulation* which takes place at the proximal end of the midrib when plants are growing under ordinary periods of daylight and darkness.

### IS THE PRESENCE OF THE AUXIN A LIMITING FACTOR IN LEAF GROWTH?

Growing leaves of plants which were kept in darkness for ten days showed evidence of tension between the midrib and the lamina. The proximal half of the midrib continued to elongate, and arched upward, while the lamina exerted a restraining influence. The result was a pronounced bulge, the upper side being strongly convex. The following explanation for such differential growth suggests itself: when plants are placed in darkness there is a complete cessation of auxin production throughout the leaf. The accumulation in the larger lateral veins and more particularly in the proximal end of the midrib enables the latter to continue to elongate as long as auxin is present in optimal concentrations for growth. Little or no growth takes place in the lamina because all of the auxin has been moved into the midrib (or being present only in low con-

centrations, it is soon exhausted in intervenous portions when the plant is placed in darkness).

To test this question, heteroauxin was applied in lanolin paste to different parts of leaves after the method of Laibach (1933). There is little or no response when the paste is put on the lamina, but when applied externally to the midrib, the latter becomes sharply convex on the side where

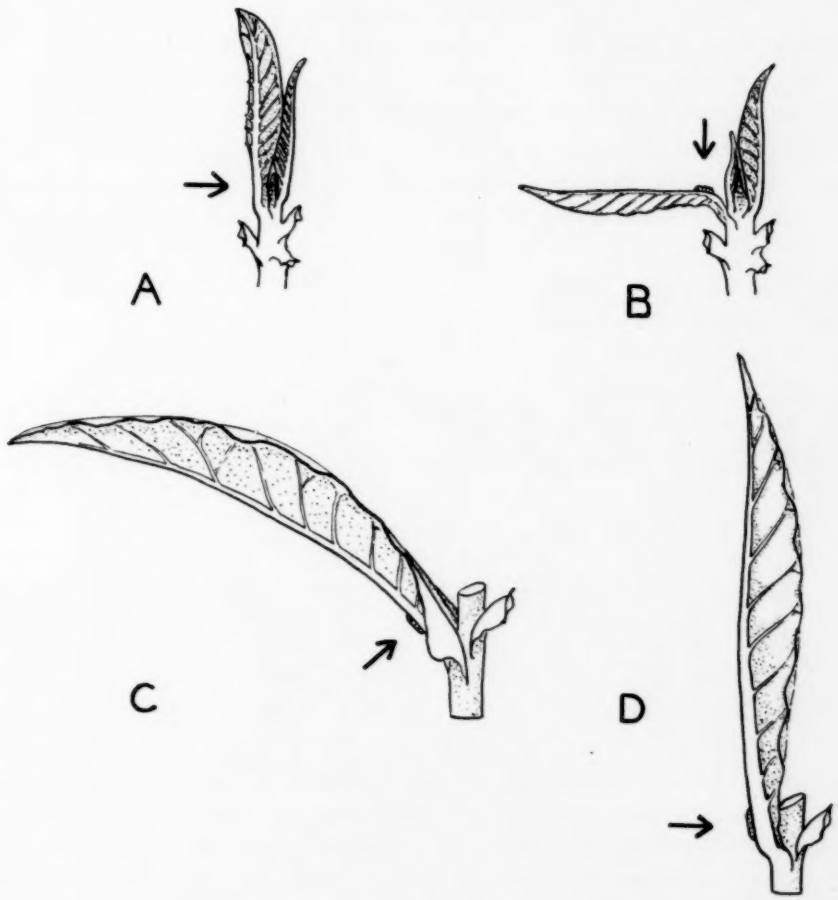



Fig. 4. Response of leaves to the application of auxin paste. A, young leaves still upright in the bud. Auxin was applied to the adaxial surface of the midrib (leaf on the left) at the level indicated by the arrow, B, same, 18 hours later. C, somewhat older leaf, on which auxin was applied to the abaxial surface of the midrib as indicated. D, same, 18 hours later. These and similar experiments were carried on in the darkroom, on plants in which the auxin was completely absent; they had been in darkness for approximately a week previous to applying the auxin.

it is applied. The bend actually occurs just below the level of application (fig. 4). The degree of response, i.e., the angle of bending, depends upon the degree of maturity of the leaf and upon the distance of the applied auxin from the proximal end. Angles up to 90° may be obtained if the paste is applied to the adaxial surface of upright young leaves at their extreme proximal end (fig. 4 A, B).

If the paste is applied to the lower (abaxial) side of the midrib at the proximal end of the leaf, the leaf will bend upward just as in the night position (fig. 4 C, D). Mature leaves do not ordinarily assume the "night" position, and so in order to determine whether age of leaf was a factor of influence, successive leaves ranging from the younger ones (starting with one 10 cm. long at the upper end of the stalk) down to mature leaves, were treated. The angle between the midrib and the internode was measured by means of a protractor before and after application. The plants had been in the dark room for 200 hours and the leaves no longer moved nor in any way changed their position from time to time with respect to the stem. The results were as follows:

| LEAF NUMBER, STARTING WITH UPPERMOST LEAF TREATED | SIZE OF ORIGINAL ANGLE AT THE TIME OF TREATMENT | SIZE OF ANGLE THREE DAYS AFTER APPLICATION OF AUXIN PASTE | NUMBER OF DEGREES MOVEMENT DUE TO THE AUXIN |
|---|---|---|---|
| 1 | 15° | 0° | 15° |
| 2 | 35° | 8° | 27° |
| 3 | 60° | 18° | 42° |
| 4 | 45° | 31° | 14° |
| 5 | 65° | 10° | 55° |
| 6 | 40° | 39° | 1° |
| 7 | 50° | 52° | −2° |

It will be noted from the table that the upper five leaves to which the auxin paste was applied showed a marked response, each leaf assuming as nearly an upright position as possible (mechanical interference with one another kept them from attaining a completely upright position). The sixth and seventh leaves which were old and becoming yellow, showed no response, a fact which falls in line with results recently reported by Mai (1934). He found that when auxin was applied to old *Coleus* petioles, they failed to react, but when applied to younger petioles they responded with greater than normal elongation.

The growth shown by midribs of younger leaves in response to the application of auxin paste indicates that the hormone is at least one of the factors responsible for growth of veins. The fact that the proximal third of the midrib is characterized by a strong *polarized growth* (fig. 1, B) and

that the auxin normally accumulates in this region, now ties up with the fact that marked growth responses take place when additional auxin is artificially applied to the midrib. That auxin paste has less effect when applied to the lateral veins may be explained by the fact that it moves rapidly into the midrib.

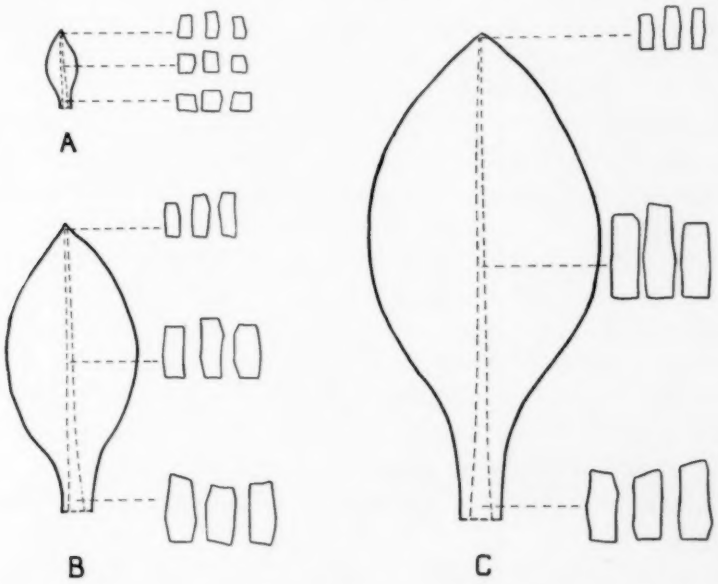



Fig. 5. Diagrams of young (A), half-grown (B) and mature (C) leaves, showing relative size of parenchyma cells in the "cortical" region of the midrib. Longitudinal sections 20 microns in thickness were made in the three zones indicated (paraffin method). Measurements of the twenty largest cells were used to compute average cell size. The range in size is indicated in the drawings. The enlargement of these cells was determined relative to the enlargement of the leaf as a whole (Huxley's "k," see text). The cells at the tip mature early. Those in the middle increase in length at about the same rate as the leaf. Those at the base increase in length about in proportion to the leaf, up to the time it is half-grown. From then on to maturity, growth is due to division and subsequent enlargement of cells; cell division in this region is correlated with a high concentration of auxin.

### DOES THE AUXIN INFLUENCE CELL ELONGATION, CELL DIVISION, OR BOTH?

The distinction between phytohormones which bring about cell enlargement and those which bring about cell division was pointed out in the introduction. While it is beyond the scope of this study to discuss the question at length, an examination of increase in cell size in proportion to leaf size was made, using Huxley's formula for heterogonic growth (Avery,

1933). Data on length and volume were obtained for cells in three distinct regions in the midrib: (1) the apex, (2) middle, and (3) base of young (3 cm. long), half grown (12 cm. long), and mature leaves (30 cm. long). The twenty largest parenchyma cells were selected from the "cortical" region of the midrib at each of the three levels in the leaves of the three different ages. It was found that the cells at the tip matured early. Those in the middle increased in length at about the same rate as the leaf, throughout the period of growth. Those at the base increased in length about in proportion to the leaf as a whole, up to the time it was half grown. From then on until maturity, in this basal region, cell enlargement did not keep up with the growth of the leaf as a whole, i.e., further growth is due to division and subsequent enlargement of cells (fig. 5). Data based on increase in cell volume in proportion to leaf volume are of the same order as the above for cells in the middle of the leaf. At the base, cell volume is increasing only 0.3 to 0.4 times as rapidly as the volume of the leaf as a whole, which corroborates the above evidence for cell division. New cells, therefore, are being added in the direction of polarized growth. It is difficult to interpret the data. The hormone may be promoting cell division. It may on the contrary be promoting cell enlargement, and the cells after reaching a certain size, divide and thus maintain a favorable nuclear-cytoplasmic ratio. In either case, there seems to be a definite relation of the auxin to polarized growth.

It is impossible at this time to say precisely what relationship exists between auxin and *localized* growth in various portions of the *Nicotiana* leaf.

### THE HORMONE PRESENT IS PROBABLY AUXIN *A*

Since (Kögl and others) auxin *a* is stable in the presence of acid and auxin *b* is sensitive to both acid and alkali, and heteroauxin is stable to alkali but destroyed by acid, it seems likely that auxin *a* is the hormone present in *Nicotiana* leaves, because in numerous experiments it was extracted with acidulated chloroform.

### SUMMARY

1. A hormone, probably auxin *a*, is demonstrated in growing leaves of *Nicotiana*. The concentration is roughly inversely proportional to the age of the leaf, that is, it is present in greater concentration in young leaves and tends to disappear as a leaf matures.

2. There is a definite auxin concentration gradient from tip to base of leaf. It is low at the distal end, and successively higher toward the base.

3. Auxin is produced in nearly equal concentrations over the entire

lamina in young rapidly growing leaves, and the gradient referred to in "2" is due to accumulation at the base.

4. Accumulation and transport of auxin take place mainly in the midrib, to which the auxin is conveyed by the large lateral veins.

5. Movement of auxin in the leaf is polar, i.e., transport takes place only from tip toward base of leaf, and from lateral veins into the midrib.

6. Auxin tends to disappear as the leaf approaches maturity, first at the apex and then gradually toward the base.

7. The formation of auxin in the leaf depends upon the presence of light.

8. Elongation of the midrib and probably the larger lateral veins is due to favorable concentrations of auxin. Growth can be induced by external application of the auxin paste to the midrib. Similar applications to the intervenous regions or lateral veins have little effect, probably because the auxin is moved rapidly to the midrib. This suggests that the impetus for leaf development may come from the midrib and main lateral veins, due to the accumulation of auxin in them.

9. Auxin is partly responsible for the growth pattern shown by the leaf: the proximal portion of the midrib (and therefore of the entire proximal end of the leaf), where auxin accumulates, undergoes relatively greater growth in length ("polarized growth") than the leaf as a whole.

Cell division is shown to be taking place in the proximal end of the midrib, where the auxin accumulates. This is contrary to the present generally accepted notion that auxins influence only cell enlargement. Auxins and their possible influence on cell division and cell enlargement are discussed briefly.

It is a pleasure to express my appreciation to Dr. F. W. Went, at whose invitation I visited the Kerckhoff Laboratories at the California Institute of Technology in the summer of 1934. With similar appreciation I wish to convey my thanks to Dr. K. V. Thimann, Mr. Folke Skoog and Mrs. Franz Dolk, for their assistance and helpful discussions.

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# The natural distribution of Cytisus scoparius in Virginia with special reference to soil reaction[1]

THOMAS W. TURNER

## INTRODUCTION

While studying the flora in certain sections of tide-water Virginia, my attention has been directed frequently to *Cytisus scoparius*, or Scotch broom as it is generally known. Its localized distribution and the apparent indifference as to kind of soil in which it is seen to grow, prompted me to make some studies for the purpose of determining the extent of its distribution over the state and at the same time to investigate the reaction of the soil from which the roots take their nutrients. The absence of seedling growth, in and around places of luxuriant adult stands, and the almost weedy appearance of the same in other places not far removed from these stands, have led to experiments, also, to determine to what extent germination of seeds is influenced by the hydrogen ion concentration, and to show further whether or not the germination of the seeds requires a soil reaction different from that in which roots are found growing most abundantly.

Attention is further called to this plant because of the showy yellow flowers, which in massed aggregations, present a beautiful scene along the roadside in late spring and give much promise from the point of view of the nurseryman and florist.

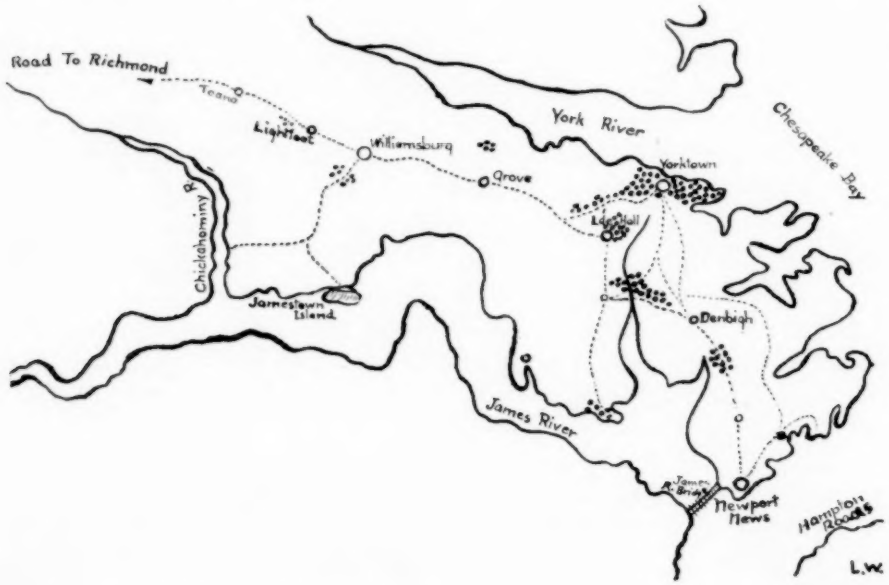
It was in 1884 that a French landscape gardener by the name of André noted a seedling differing from the surrounding *Cytisus* plants in having crimson wing petals; he selected this and from its propagation have been derived, chiefly through efforts of European gardeners, several well known commercial varieties. American ornamental plant growers have given but little attention to the common variety of broom which has become naturalized in several restricted localities in different parts of the country. It has many commendable natural features, among which may be mentioned the abundant floriferous habit, extending over a rather long period, the large bright yellow flowers which are very attractive against a background of dark green twigs and foliage, the comparative hardiness and ease of propagation from seeds, as well as from cuttings.

## DISTRIBUTION AND LITERATURE

While *Cytisus* is a well known wild plant, sometimes considered a troublesome weed, in Great Britain and parts of the continent of Europe,

[1] Contribution from the Department of Biology, Hampton Institute, with cooperation of the Virginia Academy of Sciences, Richmond, Va.

it has become established in the United States and Canada slowly and only along or near the coast line. This is true of the Pacific as well as the Atlantic Coast. Gray's *New Manual* lists it as occurring on "sandy barrens, etc., Nova Scotia; south-east, from Massachusetts to Virginia and southward." Britton and Brown's *Flora* gives the distribution as follows: "In waste places, Nova Scotia to Massachusetts, Delaware and Virginia, also in California and on Vancouver Island." It might be added that along the Pacific Coast it is to be found in the States of Oregon and Washington as well as California.




Map of the Peninsula Section of Virginia. The distribution of *Cytisus scoparius* over the Virginia Peninsula.
Broken lines—auto roads
Dots—*C. scoparius*

The Virginia distribution, with which this study deals, is quite typical of the general distribution as we see it on this side of the Atlantic. There is no extensive nor gradual invasion of new areas at any place. Thus, it would seem that the dotted growths as shown on the map, the abrupt mature patches, observed in places, and the springing up of seedlings in certain areas recently burnt over and cleared of trees, cannot be explained in a simple manner. There are certain barriers to the distribution of this plant which probably deserve more attention than has been given to them heretofore.

The plant is decidedly heliophilous and, in the Peninsular area, tends to cling near the roadside, the explanation of which is obvious since frequent cultivation of the cleared places serves to eliminate it before it has time to make a stand.

There is much speculation as to the origin of seeds giving rise to the first local invasions; the prevailing tradition attributes it to seeds in the horse feed and bedding imported during the Revolutionary Campaign about Yorktown. A further tradition is that the Campbell, Albemarle, and Bedford County growths originated in seeds brought from Europe and planted by Thomas Jefferson. The distinguished Virginian maintained homes in the latter two counties.

The soil reaction of *Cytisus* has been studied by Professor E. T. Wherry of the University of Pennsylvania. He places it in a group, having a pH ranging from 5 to 6.5. As my observation showed it to be growing rather freely on stiff red clay banks along the side of the roads as well as in the more usual sandy loam, a check up on the root soil reaction was deemed advisable. Such was later undertaken, though not yet completed, for germination of the *seeds* also.

It should be said here that the extreme western part of the state has not been visited in the study; thus reservation is to be made for any omission concerning this section.

## METHOD AND RESULT

In checking up the localities of naturalized invasions of broom, I have had the helpful assistance of the Federal Farm and Home Demonstration agents in the several counties of the state, who pointed out any growths known to them. These localities were then followed up for personal observation and study. Samples of soil were taken from the shallow roots which usually are found most abundant from one to three inches below the surface. These samples were carried to the laboratory in cleaned, stoppered bottles, sometimes in manila bags, for the determinations. The number of samples taken from the different areas vary as shown in the table on the following page.

Careful pH determinations were made of about 200 samples of soils collected sometimes from as many as fourteen different patches in a particular locality covering the four Peninsular counties. Determinations were also made of samples from other parts of the State (Campbell, Bedford, Prince Edward, and Amelia Counties) in which broom has been found growing luxuriantly in a wild state.

The soil was extracted by using one part to four of distilled water and

*pH value of soil around nutritive roots of Cytisus scoparius*

| SAMPLE | TRIP 1 | | TRIP 2 | | TRIP 3 | | TRIP 4 | | TRIP 5 | | TRIP 6 | | TRIP 7 | | TRIP 8 | | TRIP 9 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SOIL | pH | SOIL | pH | SOIL | pH | SOIL | pH | SOIL | pH | SOIL | pH | SOIL | pH | SOIL | pH | SOIL | pH |
| 1 | SaL | 6.2 | L | 5.8 | C | 6.0 | * | 6.8 | SaL | 7.2 | SaL | 6.8 | Sa | 6.2 | * | | L | 6.2 |
| 2 | SaL | 5.8 | L | 6.0 | C | 6.2 | | 6.8 | SaL | 7.2 | SaC | 6.3 | C | 6.3 | | | L | 6.2 |
| 3 | SaL | 6.2 | C | 6.5 | C | 6.2 | | 6.8 | SiL | 6.2 | SiC | 6.7 | L | 6.5 | | | CL | 6.2 |
| 4 | SaL | 6.8 | C | 6.4 | CL | 6.2 | | 6.8 | SiL | 5.9 | L | 6.5 | SiL | 6.5 | | | L | 6.0 |
| 5 | SaL | 6.6 | Si | 6.3 | CL | 6.4 | | 6.8 | L | 6.0 | Sa | 6.5 | SaC | 6.4 | | | L | 6.2 |
| 6 | SaL | 6.6 | SiC | 6.3 | CL | 6.4 | | 6.0 | L | 6.6 | Sa | 6.7 | SaC | 6.7 | | | SaC | 6.2 |
| 7 | SaL | 6.6 | SaL | 6.3 | CL | 6.4 | | 6.6 | C | 8.2 | L | 6.7 | SaC | 6.0 | | 6.0 | | |
| 8 | SaL | 6.4 | SaL | 6.3 | CL | 6.6 | | 6.6 | C | 8.3 | L | 6.8 | SaC | 6.2 | | | | |
| 9 | SaL | 6.4 | | | CL | 6.6 | | 6.8 | Sa | 6.7 | SaC | 6.4 | SaL | 6.1 | | | | |
| 10 | SaL | 6.4 | | | CL | 6.6 | | 6.8 | SaL | 6.2 | SaC | 6.5 | SaL | 6.8 | | | | |
| 11 | SaL | 6.4 | | | CL | 6.6 | | 6.0 | CSa | 6.3 | SiC | 6.4 | SaL | 6.8 | | | | |
| 12 | SaL | 6.2 | | | C | 6.2 | | 6.0 | CSa | 6.2 | SaC | 6.5 | Sa | 6.6 | | | | |
| 13 | | | | | C | 6.0 | | 6.0 | CGr | 6.6 | SaL | 6.6 | C | 6.7 | | | | |
| 14 | | | | | C | 6.0 | | | CGr | 6.6 | SaC | 6.4 | L | 6.5 | | | | |
| | Aver-ages | 6.4 | | 6.2 | | 6.3 | | 6.6 | | 6.7 | | 6.6 | | 6.5 | | 6.0 | | 6.2 |

Legend: C—Clay; Gr—Gravel; L—Loam; Sa—Sand; Si—Silt.
* Not recorded.

shaking vigorously. The aqueous mixture was allowed to stand, for clearing and settling of soil particles, usually five to twelve hours. The determinations were made, for the most part, by the colorimetric method, using the La Motte Testing Set Model B for the same. As a check upon these results, however, a quinhydrone electrode apparatus was used on a few random samples.

The results of the soil determinations (colorimetric method) are given in the table. This table shows a set of samples, as many as fourteen in some cases from each patch, taken from all the patches of naturalized growths found in the Peninsular section and five from areas much farther inland. No attempt is made here to present a minute and technical description of the soil. The simple classification used is based upon Emerson's *Principles of Soil Technology*. The pH values of the samples from the different areas of the nine localities are presented in the table with averages of the same. Attention should be called, however, to two samples (7 and 8 in trip 5) of clay with pH values of 8.2 and 8.3. The plants appeared normal in this highly alkaline soil. It is possible that the roots from which the soil was removed were not those functioning chiefly in the nutrition of the plants. Checking up determinations made with the quinhydrone electrode apparatus, showed a range that was in general quite

similar in pH (ranging from 6.00 to 6.7) to the colorimetric. For the electrometric measurements certain random selections were made from samples whose pH had been determined first by the color method. As shown in the table, samples 4, 5, and 6 of trip 1 gave pH values 6.8, 6.6 and 6.6; for these same samples electrometric measurements gave 6.05, 6.00 and 6.4 respectively. Trip 4, sample 4 gave, on the colorimetric basis, a pH of 5.9, the electrometric measurement gave the same figure (5.9). For the, apparently, abnormal sample 7, trip 5, the color measurement was 8.2, the electrometric measurement for this was likewise 8.2.

## DISCUSSION AND CONCLUSION

Dr. Wherry lists this plant among those preferring a subacid soil—pH 5 to pH 6—growing also in mediacid soil having a mid value of pH 4.5 to what he designates a high miniacid soil with a mid value of pH 6.5. It would seem from the soil tests made during the present study that its pH tolerance range is much narrower than Dr. Wherry's list would indicate and its acid preference much less pronounced. Instead of having a range from 4.5 to 6.5 it appears to show a decided preference of a range from about 6 to 6.7.

To what extent the narrower range found becomes a limiting factor in the distribution of the plant cannot be determined with any definiteness from the facts in hand, but certainly in presence of such range its slow natural distribution would be expected wherever a widely varying soil reaction is encountered. A comparison of the reaction of nearby soils which had remained untilled for some time was not made but might have been instructive.

Cultivation practices must also be taken into consideration as a limiting factor of no small importance in the areas under discussion. Thus, when these two factors are judged in connection with a reduced chlorophyllous mechanism that makes *Cytisus* seek the open places, we may get some clue as to its peculiar distribution.

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## A mislaid mistletoe

WILLIAM TRELEASE

In February, 1934, my long-time friend, Professor C. Conzatti, of Oaxaca, sent me specimens of a *Phoradendron* collected by him and a colleague in June, 1906, and again in March, 1909, by himself and one of his sons, both in the mountains of the Mexican State bearing the same name.

Both specimens were labeled *P. auriculatum*, a manuscript name that I must have furnished in some way while preparing my monograph of the genus *Phoradendron*. This binomial, without indication of author, appears in the list of species excluded from the genus (p. 217); but, unlike other excluded species—with a single exception, without indication of an accepted name, and it appears also in the index to specific names with a quaere as to its application (p. 219).

Professor Conzatti's curiosity as to where I had seen and so labeled the plant, was fully equaled by my own, coupled with wonder concerning its disappearance except as an excluded name from a monograph published nearly a decade after it had been collected first. No specimens occurring in my own collection, an appeal was made to the Missouri Botanical Garden, where all of my earlier material is deposited; and, no trace of it being found there, to the United States National Herbarium, in which the genus had been studied, but where nothing of the kind occurs.

Preoccupation with other matters, and a rather prolonged vacation in the summer, prevented me from making further search for the missing species until recently.

A more thorough search now reveals the fact that I first encountered it in the Gray Herbarium, probably on my return from Europe early in 1913, at which time the appended manuscript description was drawn up under the questionable name. When my notes were arranged in monographic form, two or three years later, though *P. auriculatum* found its way into the index without being placed and for this reason into the list of excluded names, the description drifted out of sight. Now that it has come to light again, the reason for its exclusion appears to have been a doubt as to the availability of the proposed name, and neglect either to clear it of suspicion or replace it by an acceptable name before the monograph was published. The seeming difficulty of that time not being evident to me at present, the really attractive species is now given belated publication, with apologies to its talented discoverer.

**Phoradendron auriculatum** n. sp.

(Aequatoriales Amplectentes)

Somewhat pseudo-dichotomous; dioecious?; smooth and varnished, with sharply 2- or 4-angled internodes. Cataphyls confined to the lowest joint, solitary, basal, tubular; leaves subelliptical, 20–30×50–90 mm., sessile and auriculate, obtuse, sometimes slightly mucronate, rosy-margined, thick, prominently 7–9-nerved from the base but scarcely veiny. Spikes mostly

clustered, rather long (becoming 50–60 mm.) with about 4 fusiform joints some 30-flowered in 6 ranks; peduncle stout, scarcely 5 mm. long; scales spreading truncate, scarcely ciliate; immature fruit slightly cellular-papillate, globose, 3 mm. in diameter, with closely inflexed sepals.

Cordilleran region of Mexico; Cerro Nueve Puntas, Chiconahiultepec, Matatlan (Conzatti and Vasquez 1511). El Parian, Etla (Conzatti and Son 2378).

## Atractobasidium, a new genus of the Tremellaceae

G. W. Martin

(with two figures)

The fungus which is the subject of this note was collected by C. L. Smith in Mexico some time during the years 1894 to 1896. It has been in the collection of the State University of Iowa under the name *Corticium nigrescens* Fries, as No. 98 of a miscellaneous series of collections supplemental to Smith's Central American Fungi. So far as known it was not distributed and I assume that our material, which fortunately is ample, represents all that was gathered.

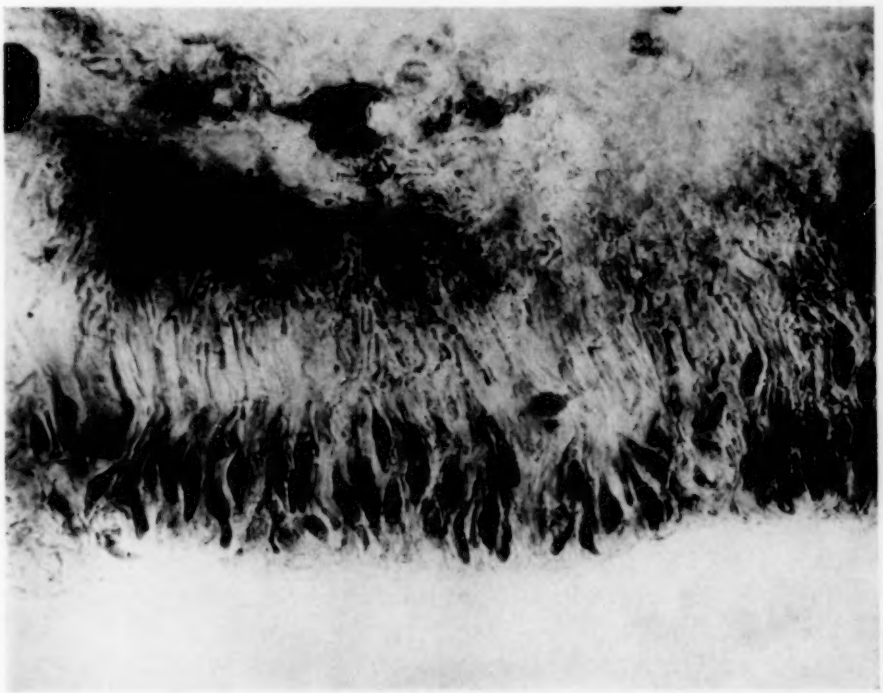

Fig. 1. Photomicrograph of freehand transverse section of fructification, stained with phloxine, ×450.

Examination of the hymenium (fig. 1) shows at once that the fungus is not a *Corticium* but a heterobasidiomycete with basidia of a type not hitherto reported, somewhat intermediate between those of the *Tremellaceae* and those of the *Auriculariaceae*. It is quite impossible to include it in any previously recognized genus and a new genus must therefore be established to accommodate it.

**Atractobasidium** gen. nov.[1] (ἄτρακτος, a spindle).

Resupinate, waxy, drying horny: basidia at first clavate, then fusiform, finally divided into four cells by oblique-transverse septa, the two later septa at right angles to the first.

**Atractobasidium corticioides** sp. nov.[2]

Receptacle broadly effused, attaining 10 cm. or more in length and 3 cm. or more in width, thin, adnate, waxy, drying horny, the central portion benzo brown*[3] to Quaker drab* when dry, becoming army brown* when soaked, fading to sordid white at the thin, indeterminate margin; in section 150–300μ thick, consisting of a thin, brownish basal layer about 20μ thick, then a densely interwoven central portion composed of colorless, irregular, vesiculose and submoniliform hyphae intermingled with some brownish strands, the two layers occupying from slightly more than one-half to two-thirds the thickness of the fructification, the remainder being occupied by the hymenium, composed of closely packed, vertically arranged basidia and slender, unbranched paraphyses with tortuous, submoniliform tips, the whole immersed in a gelatinous matrix which forms a continuous layer over the surface, the entire section with rather large, subcrystalline, calcareous accretions scattered sparsely throughout; basidia at first clavate, then fusiform, 33–45×8.5–12.5μ, eventually four-celled; divided first by a transverse-oblique septum into two cells, each cell again divided by an oblique septum at right angles to the first; each of the four cells so formed producing a tortuous epibasidium of approximately uniform diameter except toward the tip, where it is often somewhat inflated, 30–40×2–3μ, tipped by a sterigma and a spore; basidiospores ovate-cylindrical to allantoid, 11–13.5×4.5–6μ.

Type: Jalapa, Vera Cruz, Mexico, 1894–1896, *C. L. Smith* **213**. The type specimen is in the herbarium of the State University of Iowa, with portions distributed to the Farlow Herbarium, the New York Botanical Garden, the Missouri Botanical Garden and the United States National Herbarium.

The original material is in a number of fragments, but when these are pieced together it becomes evident that the dimensions given in the de-

[1] Resupinatum, ceraceum, siccatum corneum; basidia primum clavata, deinde fusiformia, septata, septo primo oblique transverso, septis secundis rectis angulis primo septo adhaerentibus.

[2] Receptaculum late effusum, ceraceum, siccatum corneum, fuscum, margine pallidum; basidia fusiformia, 3-septata, septo primo oblique transverso, septis secundis rectis angulis primo septo adhaerentibus, 33–45×8.5–12.5μ; epibasidia filiformia, 30–40×2–3μ; sporae ovato-cylindraceae vel allantoideae, 11–13.5×4.5–6μ.

[3] An asterisk affixed to names of colors indicates that they are used in the sense of Ridgway, Color Standards and Nomenclature.

scription are conservative. Judging by analogy with forms of similar habit, its growth may well be much more extensive. While the general appearance is that of a *Corticium*, the texture is unlike that of any *Corticium* with which I am familiar. The basidia, of course, constitute the striking characteristic. At first nearly cylindrical (fig. 2i), when they have attained full

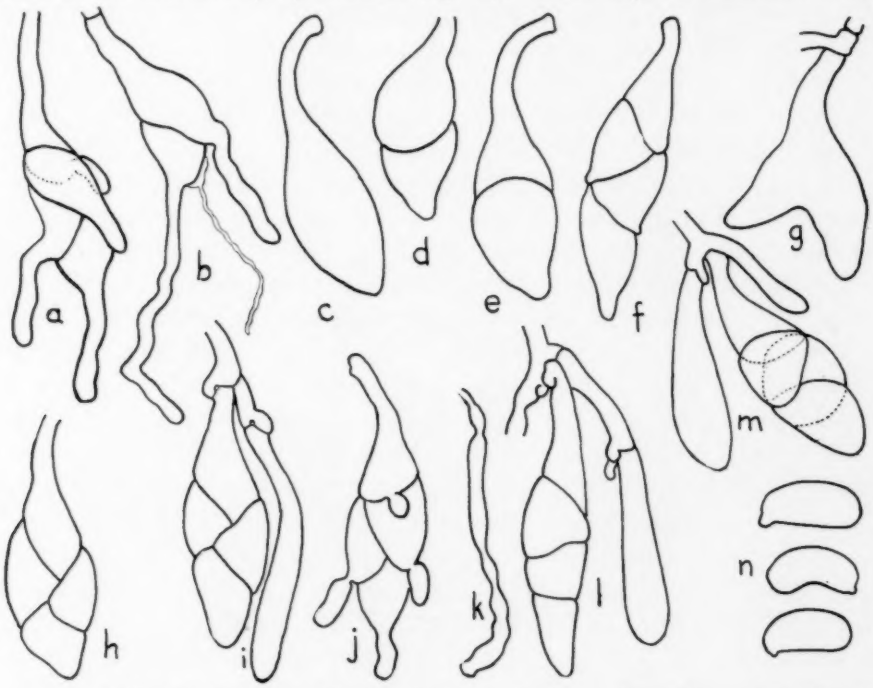


Fig. 2. Drawings made with aid of camera lucida, reduced in reproduction to ×1000. a, j. Basidia with four partially developed epibasidia. b. Basidium with two cells bearing epibasidia, one cell collapsed, fourth cell not visible. c. Mature probasidium. d, e. First division completed. f, h. Four-celled basidia, epibasidia beginning to develop in f. g. Basidium of unusual shape. i. Probasidium and four-celled basidium, showing clamp connections. k. Paraphysis. l. Probasidium and four-celled basidium, the latter with secondary walls not reaching the first septum. m. Two probasidia and fully divided basidium in cluster. n. Three spores.

size they are clavate-fusiform (fig. 2c), then, at about the time the first division occurs, the distal end becomes bluntly pointed and often somewhat attenuate (fig. 2d, e). The first division is obliquely transverse, at an angle of about 45° to the axis of the basidium, the second divisions are at right angles to the first, but on account of the shape of the basidium the secondary walls do not meet on opposite sides of the first septum, as in typical basidia of the tremellaceous type (fig. 2h, i). The second divisions must follow the first division very promptly since unseptate and four-celled

basidia are much more abundant than those with two cells. Most of the basidia are provided with prominent clamp connections at the base (fig. 2i, l, m). A few of irregular form were found (fig. 2g) but these seemed to be no more abundant than is usual in tremellaceous fungi.

The position of the first septum recalls the situation in *Sirobasidium*. The resemblance is, however, superficial only. In *Sirobasidium* the oblique position of the septum is clearly an adaptation to the catenulate arrangement of the basidia. Lagerheim and Patouillard (1892) illustrate the septa in the terminal basidium of both species they describe as strictly longitudinal, and this seems to be the case with all the basidia in the chain shown in their fig. 1f. The secondary septa are shown on opposite sides of the primary septum, as in *Tremella* and *Exidia*. The basidia of *Sirobasidium* as pictured by Möller (1895, pl. 6) and by Coker (1920, pl. 55, figs. 1, 2; 1928, pl. 47, figs. 1–4) are never more than 1-septate, and the septum in the terminal basidium is usually oblique, but occasionally longitudinal or directly transverse. Furthermore, the basidial segments of *Sirobasidium* do not produce epibasidia, but bear the basidiospores directly These differences, wholly aside from the catenulate arrangement of the *Sirobasidium* basidia and the gross habit and morphology of *Atractobasidium*, make it clear that the latter genus cannot be accommodated in the *Sirobasidiaceae*. The resemblance to certain genera of the *Auriculariaceae* with swollen, clavate, transversely septate basidia is also suggestive at first sight. One exceptionally slender basidium was observed in which the secondary septa had failed to reach the first septum (fig. 2l). Such irregularities are of common occurrence in the *Tremellales* and have been illustrated many times. An example of an irregular basidium of *Tremella lutescens* suggesting the regular type in *Atractobasidium* is shown by Coker (1920, pl. 57, fig. 4). Nevertheless, the regular, perpendicular alignment of the second basidial septa with reference to the first septum is held to exclude the genus from the *Auriculariaceae* and to establish its position in the *Tremellaceae*. It remains true, however, that the *Atractobasidium* type of basidium is more like basidia such as those of *Platygloea* and *Auricularia* than any of the tremellaceous basidia heretofore described. Rogers (1934) has recently suggested the possible independent derivation of the auriculariaceous basidium from the primitive tulasnellaceous type, or even from an hypothetical ascomycetous ancestral group. The existence of *Atractobasidium*, however, suggests possible direct relationship between the *Tremellaceae*, through that genus, with the two genera of the *Auriculariaceae* mentioned.

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# Nuclear behavior in the tapetum of Hosta caerulea, with special reference to the divisions[1]

Wm. E. Roever

(with plates 19 and 20)

## Introduction

Mitosis is commonly defined as nuclear division involving the formation of chromosomes, while amitosis is regarded as division of the nucleus in a "resting" condition, that is, without the organization of chromosomes. It was once believed that amitosis was the prevalent method in cell division and mitosis, the unusual type; but as work on nuclear division accumulated in all fields of biology, it became evident that the converse was true. Promulgation of the chromosome theory induced the continued and critical study of nuclear division in both plant and animal kingdoms. With the gradual accumulation of data, mitosis steadily encroached upon domains where amitosis was formerly thought to be the routine method of nuclear multiplication, so that at present few examples remain to be pointed out as bona fide instances of nuclear fission. In any case, it is extremely doubtful whether there exists a single case of amitosis in the sense of a division having the same reproductive potentialities as ordinary mitosis.

In reference to the problem in protozoa, Kofoid (1923) says, "Amitosis as described in protozoa is either a pathological or degenerative process as it is in metazoa." Among fungi, the budding of yeast, long regarded as a classic example of amitosis, has been distinctly figured and described as a case of mitosis (Kater, 1927); and in the higher plants described instances of nuclear fission have, in general, occurred in degenerative or pathological tissues, or have been found to be due to incomplete mitosis or fusion.

Among the plant tissues that have received much study in connection with this problem of nuclear division has been the staminal tapetum. Conflicting reports concerning the methods of division in the tapetum suggested an investigation of nuclear behavior during the development of this nutritive layer.

The tapetal cells in the anthers of most pteridophytes and spermatophytes are characterized by a multinucleate condition that appears early in the development of the pollen sacs. The origination of this multinucleate phase has variously been described as either mitotic or amitotic, and one author reported that both methods of division occur.

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After the polynucleate stage has been attained the tapetal layer undergoes changes which are closely correlated with microsporocyte development. Such changes are markedly evident in the behavior and appearance of the nuclei and culminate in tapetal degeneration coincident with the formation of pollen.

## HISTORICAL

The following investigators record mitosis in tapetal cells: Strasburger (1882), Bonnet (1912), Gates and Rees (1921), Campin (1925), Mascré and Thomas (1930), Thomas (1931), Steil (1933), Cooper (1933) and Smith (1933). On the other hand, Tischler (1906) speaks of both mitosis and amitosis in the tapetum of certain *Ribes* hybrids. O'Neal (1920) records amitosis in the same tissue of *Datura Stramonium*. Meyer (1925) speaks of polyploid tapetal nuclei that arise by splitting of chromosomes without loss of the nuclear membrane. Conard (1926, 1928), in wound tissue of *Tradescantia* stems, describes amitosis followed by reunion of the amitotic segments and subsequent division of the nucleus by mitosis.

As for animals, Macklin (1916) records amitosis in the living tissue of the embryo chick but Lewis and Lewis (1924) believe that most, if not all, amitosis in tissue culture has been observed in degenerative or pathological material. That artificial fission of a nucleus can be followed by reunion of the parts and subsequent mitotic division of the reformed nucleus is shown by Chambers (1917). Conklin (1917), in cleavage divisions of *Crepidula plana*, records only anomalous mitoses simulating nuclear fission.

## MATERIALS AND METHODS

Buds of *Hosta caerulea* Tratt. in all stages of development were taken from plants growing in the vicinity of New Brunswick, New Jersey. The material used in this study was collected in the latter part of June, and for the most part, very young buds were found to give the required stages.

In fixation tips of buds were cut off to allow quicker penetration of the anthers, and air was always exhausted, as the material did not readily sink in the fixing fluids because of air entrapped in the buds. Various fixing agents were tried such as Carnoy's fluid, chrom-osmic-acetic, and Allen's modification of Bouin's fluid "$PFA_3$." Only the last gave satisfactory results. It was found that the action of Carnoy's was too rigorous and induced excessive plasmolysis. Chrom-osmic-acetic fixatives, on the other hand, caused the densely cytoplasmic tapetum to appear too opaque for satisfactory observation, and the opaqueness was found to persist after subjecting the sections to a thirty-three per cent solution of hydrogen peroxide in seventy per cent alcohol for as much as twenty-four hours.

In "$PFA_3$," material was fixed for twenty-four hours and washed the same length of time. Smear preparations were then made in an effort to find some relation between bud size and microsporocyte development. Belling's aceto-carmine method gave excellent results. The stain was prepared by boiling an excess of powdered carmine in forty-five per cent glacial acetic acid. Anthers were then teased apart with a pair of steel needles and mounted in this solution. The various stages in pollen mother cell development were thus obtained and correlated with bud size. This approximate correlation proved to be of value in selecting buds for sectioning with the view of obtaining specific stages in microsporocyte development.

In the preparation of sections the paraffin method was employed. Both transverse and longitudinal sections of the buds were cut, but the latter proved by far the most useful. Most ribbons were cut at five microns and sections were mounted serially. In staining, Heidenhain's iron-alum haematoxylin was used, and in some cases a light counter stain of orange G, in clove oil, was applied.

## OBSERVATIONS

### *The resting nucleus*

At the primary sporogenous stage of an anther its wall consists of four layers, the epidermis, the immature endothecium, a transition layer, and the tapetum adjoining the primary sporogenous tissue. The young tapetal cells are square or oblong in outline, but as the anther enlarges, growth stresses force them into various angular forms. The nuclei are slightly hyperchromatic, but as the tapetum develops they become increasingly rich in chromatin.

When the microsporocytes are in the resting stage the tapetal cells are very small as compared to their size at maturity, and the single large nucleus occupies about half a cell. At this period the appearance of the resting nuclei is quite distinct. In general, they are spherical to oval in shape and contain from one to several hyaline vesicles each of which has a prominent, densely staining nucleolus. When a single vesicle is present it occupies a large part of the nucleus and contains a large nucleolus (fig. 1). If more than one is present, the vesicles are correspondingly smaller and have less prominent nucleoli (figs. 12a, 12b). Careful focussing reveals several fine supporting strands running from the periphery of a vesicle to its nucleolus (fig. 1). If the nucleoli are small these strands are not easily detected. Slight accumulations of chromatin are seen bordering the vesicular region, these being especially noticeable where two vesicles are ap-

pressed. The remaining chromatin of the resting nucleus forms a thin reticulum lining the nuclear membrane.

The foregoing description is also characteristic of the tapetal nuclei during the synizetic stages of the pollen mother cells. While synizesis is in progress the first mitotic divisions begin in the nutritive layer.

As the anther develops the tapetal nuclei gradually undergo a considerable change, and by the time the pollen mother cells have reached the open spireme stage the nuclei are decidedly more hyperchromatic than during the earlier periods of growth. Most of the tapetal cells have at least doubled their earlier dimensions and the nuclei have enlarged correspondingly (fig. 3). As a result of the first mitotic division many of the cells are binucleate; some are uninucleate because the division was incomplete or because it was accompanied by cytokinesis, while others may be uninucleate because the first division has been delayed. The large resting nuclei have, for the most part, lost the nicely rounded or oval shapes that characterize them during and before synizesis. Nuclei resulting from incomplete first divisions are often dumb-bell-shaped or U-shaped (figs. 11a, 11b, 11c), and where a pair of nuclei are present, they, too, frequently have irregular outlines.

The internal condition of the nuclei is also considerably modified. Instead of the few prominent hyaline vesicles of earlier stages one finds smaller but more numerous vesicles (fig. 11c); as a rule ten or more are present. These are often considerably masked by the chromatin content of the nuclei. In favorable cases, tiny spheres of chromatin (chromocenters) are seen attached along the nuclear reticulum (figs. 3, 11b). The whole organization of the nuclei is considerably obscured by flocculent and granular accumulations of chromatin along the nuclear reticulum. By and large, the irregular and glutted appearance of the nuclei, even at this early stage of development, gives the impression of a lack of physiological control. The condition is comparable to what is observed in many pathological or degenerative tissues. That functional activity is affected is shown by the mitotic irregularities that are prevalent. These will be described presently. The appearance suggesting functional disability of the nuclei becomes evident coincident with the rapid increase in size of the tapetal cells, and seems to be correlated with the accumulation of nutritives to be used in pollen maturation. This appearance of the resting nuclei, which becomes evident at the spireme period of the microsporocytes, remains essentially the same until tapetal degeneration which, in normal anthers, occurs about the time of tetrad formation. Such changes as do take place merely emphasize conditions that have already manifested themselves during the spireme stage.

Although breakdown of the tapetal cells usually occurs shortly after the tetrads are formed this is not always true. In some instances a premature degeneration is found as early as the spireme stage of the pollen mother cells; this may take place in a portion of a locellus only. Such breakdown always includes the microsporocytes in the affected locellus or portion of a locellus. A mechanical or physiological injury is apparently involved. In these cases of early degeneration the behavior of the tapetal nuclei is quite constant. Resting nuclei stain as dense, black spheres having a granular surface (pyknosis) (fig. 4); these finally fragment into globules of various sizes (fig. 5) and disappear (dissolve?). Other nuclei are caught in various stages of division; they, too, stain heavily and ultimately disappear by a similar fragmentation as in the preceding case.

In normally developing anthers tapetal breakdown usually begins shortly after tetrad formation. Vacuoles form at two opposite ends of a tapetal cell and gradually enlarge. The cytoplasm begins to take the haematoxylin stain to a slight degree; but the nuclei do not stain as heavily as in earlier periods of growth; they become vacuolate and their boundaries reach a maximum of irregularity characterized by lobations and ragged edges (fig. 30). Growth of the microspores rapidly crushes the tapetal cells and releases the nutritive fluid which flows into the locellar cavity where it is seen as a pale yellow substance surrounding the microspores. The crushed tapetal layer appears as an irregular black line in which an occasional dark nuclear mass is to be distinguished. Some locelli contain sterile pollen only. In these, the tapetal cells persist beyond the normal time because the microspores do not enlarge; consequently the tapetum is not crushed, and no tapetal fluid is to be found in the locellar cavities.

### *The nuclear divisions*

All observed cases of nuclear division in the tapetum of *Hosta caerulea* were mitotic. Amitotic appearances are readily explained on the basis of aberrant mitoses, vesicular appearances of nuclei, close appression of two nuclei, or degenerative fusions. Cytokinesis does not typically accompany divisions of tapetal nuclei and the cells are consequently multinucleate. Occasionally cytokinesis does occur, and wall formation may be either anticlinal or periclinal; if the former, it increases the size of the locellus; if the latter, the tapetum becomes two cells in thickness at the point of division. Mitoses occurring within a given multinucleate cell are always simultaneous (figs. 18, 25, 26).

During the resting stage of the microspore mother cells the tapetal cells are uninucleate and no mitotic figures are to be seen. At this time the vesicular nature of the nuclei frequently simulates amitosis or fusion

(figs. 12a, 12b). But with the beginning of synizesis the first mitotic divisions of the tapetal nuclei become evident, and by the time maximum synizetic contraction is reached many of the cells are binucleate or the first division is in progress (figs. 2, 8). When division is normal the daughter nuclei nearly always become closely appressed (fig. 6). Each of these appressed nuclei clearly maintains its identity, and no fusion is observed while the nuclei are in the resting stage.

At times the first mitosis is incomplete and a chromosome bridge is left connecting the daughter telophase groups (fig. 7); the resulting nucleus is presumably tetraploid (figs. 3, 7, 9, 10, 11a, 11b, 11c) and often presents an organization easily misinterpreted as the result of fusion or amitosis; dumb-bell-shaped or U-shaped nuclei are the commonly formed types. Tripolar spindles are seen occasionally (fig. 23). The exact distribution of chromosomes in such cases is problematical and can only be estimated by the size of the daughter chromosome groups, for the clumping of chromosomes that occurs in the tapetum makes accurate counting impracticable. At any rate, such division involves the apportionment of the whole chromosome complement of the cell among the resulting daughter nuclei. The number of mitotic aberrations occurring during the period of the first division is small as compared to the irregularity occurring in subsequent development.

Second divisions of tapetal nuclei begin during the spireme stage of the microsporocytes and are to be found extending into the metaphase stage of the pollen mother cells. Delayed first divisions, and divisions in diploid cells resulting from previous cytokinesis are also observed during this period. Therefore, one finds first and second divisions occurring side by side. The latter may occur in several ways most of which are aberrant. The chromosomes of two closely appressed nuclei in a binucleate cell may unite after the disappearance of the nuclear membranes in the late prophase and form a single metaphase plate (figs. 15, 16). Subsequent division, if carried to normal completion, will give rise to two nuclei that are tetraploid; if a chromosome connection is retained, a single octoploid nucleus will result (fig. 13). Sometimes the connection between daughter chromosome groups is very marked due to almost complete failure of the separatory process (figs. 24, 28). In some instances two normal metaphase spindles are observed (fig. 25), and subsequent completion of their division gives rise to four diploid nuclei (fig. 29). Such tetranucleate cells are infrequently seen. If two metaphase figures are so oriented in a cell that two of their spindle poles coincide (figs. 18, 26), a pair of diploid nuclei and one tetraploid nucleus will result, provided that each division is complete (figs. 21, 27). If one of the divisions is incomplete (fig. 19) the two resulting

nuclei will be hexaploid and diploid respectively (fig. 20). Should both divisions retain chromosome bridges a single octoploid nucleus would result; this condition was not observed to occur. Occasionally one finds trinucleate cells in which one of the nuclei is very small. Such dwarf nuclei, it seems, are produced by the segregation of a few chromosomes during an otherwise normal anaphase (fig. 14). Tripolar spindles are also found during the second division (fig. 22), and in such cases an octoploid number of chromosomes is distributed among the three telophase groups. At times the orientation of divisional figures is so enigmatic as to make the outcome purely speculative. Such appearances are especially evident during diakinesis (fig. 17), and they serve to emphasize the divisional irregularities that may occur. In one case three metaphase groups were observed in a cell. As a general statement it may be said that the later the occurrence of a division, in the plant studied, the greater is the tendency for irregularity in chromosome distribution.

Just preceding the time of final disintegration the tapetum is composed of cells whose nuclei vary from the diploid to the octoploid condition. There are cells containing single diploid nuclei; others have two. Some may have single tetraploid nuclei; others, a pair. There are cells with a single octoploid nucleus, or a hexaploid and a diploid nucleus. In short, a wide difference in nuclear valency is found; and irregular mitosis plays a large part in creating these conditions. Cells containing diploid nuclei are doubtless present because cytokinesis accompanied nuclear division; such cell division is occasionally observed. Cells having single tetraploid nuclei may result similarly, if cytokinesis is present during the second division, or if a second division fails to materialize subsequent to an incomplete first division. The previously described aberrations in the mitotic process can largely account for the remaining cases of polyploidy, and for the variations in nuclear size and shape.

In the aged tapetum fusions of degenerating nuclei occur, and these are also involved in causing variations in nuclear size and shape. Several observations of fusion occurring between nuclei of adjacent cells show definitely that degenerative fusions of resting nuclei are present (fig. 31). No fusions of resting nuclei are seen previous to this stage of final degeneration. Occasional nuclear fragmentation is also observed in the disintegrating tapetum.

## DISCUSSION

In reviewing the literature of amitosis it soon becomes apparent that wherever investigators have claimed the occurrence of amitotic division the cells involved were either highly specialized in character, ephemeral,

pathological, or degenerative. Furthermore, in nearly all verified cases of amitotic division there has been no accompanying cytokinesis. One marked exception is reported in lymphocytes (Wilson, 1925) in which cytokineiss reputedly accompanies amitosis. Even here, however, the dominant mode of division is mitotic, and amitosis appears to be rare. It is noteworthy that such cells are of a highly transitory nature; and therefore observed cases of amitosis may, even here, be occurring in degenerating cells. The problem in lymphocytes seems worthy of further investigation. It appears significant that all verified amitosis has occurred in such highly differentiated tissues, and equally significant that it has not been verified in reproductive or meristematic cells.

In view of the preceding statements it is seen that the question of amitosis seemingly concerns itself wholly with nuclear division in highly specialized cells—cells that have an *extremely* restricted line of development. Consequently it cannot, it appears, be regarded as having genetical significance.

The tapetum is one of those highly specialized, ephemeral tissues with which the amitotic mode of division has often been associated; and it would seem that divisional conditions prevalent there might reasonably serve as an indication of divisional methods prevailing in other highly differentiated, multinucleate cells, especially those having a similar nutritive function.

In the tapetum of *Hosta caerulea* the first amitotic appearances occur during the uninucleate stage when the vesicular structure of the nuclei often simulates nuclear budding, or fusion. But that neither of these occurs is shown by the consistently uninucleate character of the cells at this stage. The next period at which the tapetal nuclei falsely suggest amitosis follows immediately after the first division. Those nuclei in which complete division occurs are closely appressed, and those resulting from incomplete division are, in one way or another, connected by a chromatin bridge. Either appearance might easily be mistaken for amitotic division, especially if viewed in isolated cases; but a study in the developmental sequence of the cells involved reveals the true origin of such appearances. It is suggested that many reports of amitosis in the tapetum have resulted from observations made without a complete study of the tapetal development from its youngest stages to its maturity.

The first nuclear divisions occur in the tapetum before it has assumed its truly hyperchromatic appearance, but by the time second divisions begin the cells have markedly increased in size and the nuclear chromaticity has likewise increased. It seems that the tapetum has by this time really begun its activity of storing food to be released at tetrad formation.

These second divisions are of a remarkably aberrant nature. It appears that accumulation of nutritives within the tapetal cells initiates these divisional irregularities. Precisely what the causative factor may be is questionable, though it is perhaps bound up with changes in the density of the cell contents due to food accumulation. Increase in density within the cells might retard the separatory mechanism of mitosis.

The richly chromatic nature of the normal tapetal nuclei is also apparently bound up with the accumulation of food in the cells. This view is supported when it is recalled that very young tapetal nuclei are lacking in hyperchromaticity, undoubtedly because the cells have not yet begun the accumulative processes which so modify their structure. Farmer and Digby (1910) showed, in certain ferns, that tapetal cells which have lost their secretory function have wholly normal nuclei that are lacking in richness of chromatin.

Chromocenters begin to appear in the nuclei just as soon as the tapetal cells start their rapid enlargement and storage. The appearance of chromocenters, too, is apparently linked with the amassing of food in the tapetal cells. Tischler (1921–1922) cites cases where hyperchromaticity and production of chromocenters result from "feeding" tissues with a peptone solution. Such chromatin richness disappears as soon as "feeding" is arrested.

As previously stated, the second divisions occur after the tapetum has become food laden, and this is believed to be the main factor causing incomplete mitoses. In addition, the presence of more than one nucleus within a cell frequently results in a coinciding of spindle poles, and a consequent fusion of two telophase groups, coming from separate nuclei, to form a polyploid nucleus. Such fusions are dependent upon the orientation of the two divisional figures within a cell. Trinucleate cells that result in this manner show one nucleus approximately double the size of each of the others. The inequality of nuclear size in such cells might easily lead one to infer that they were caused by nuclear budding. When an incomplete mitosis accompanies such a "fusion division" (fig. 19) the resulting nuclear appearance is extremely difficult to interpret from mere observation of the resultant resting nuclei (fig. 20). Similar conditions have, at times, doubtless been labelled "amitosis." After most of the second divisions have occurred in the tapetum one finds few cells containing four diploid nuclei. Absence of such tetranucleate cells clearly indicates that few cells complete the two successive divisions in a normal manner to produce a tetranucleate condition. In this connection, however, it must be remembered that cytokinesis may accompany some of these divisions and thus account for the absence of tetranucleate cells in certain cases.

Late appearances simulating amitosis are found in the aged tapetum and are the aggregate result of irregular mitoses combined with degenerative fusions. The nuclei strongly suggest amitotic budding. Appearances at this stage are such as might easily be misinterpreted by the casual observer, but again developmental study clarifies the conditions prevailing. Disintegrative fragmentation of nuclei is occasionally seen at this time and is the only semblance of amitosis to be observed during tapetal development.

## SUMMARY

1. Nuclear divisions in the tapetum of *Hosta caerulea* are typically mitotic.

2. No amitosis was seen, excepting occasional fragmentation accompanying nuclear disintegration in aged tapetal cells.

3. Mitotic divisions are highly irregular. The irregularities appear as: incomplete mitoses giving rise to polyploid nuclei, fusions of two prophases to form a single large metaphase plate, fusions of two telophase groups due to converging spindles from a pair of metaphases, and formation of multipolar spindles.

4. Irregularity of mitotic division increases as the tapetum becomes older and reaches its height during the diakinesis period of the microsporocytes.

5. Increased divisional irregularity appears to be correlated with the increase of nutritive material within the tapetal cells, and is seemingly caused by some physiological disability accompanying this accumulation of foodstuffs.

6. Fusions of chromosome groups during divisional processes are dependent upon the orientation of the mitotic figures within the cell; if spindle poles coincide, fusions result.

7. Few tetranucleate cells are seen, indicating that complete regularity through the two successive nuclear divisions occurring in many tapetal cells is the exception.

8. Mitotic irregularities account for amitotic and fusion appearances, and also for variations in nuclear size within a single cell.

9. Degenerative fusions in the aged tapetum also frequently assume amitotic appearances. Such fusions may occur between the nuclei of adjacent cells.

10. The vesicular structure which tapetal nuclei show during the resting stage of the pollen mother cells often simulates amitosis or fusion.

11. The rich chromatin content of tapetal nuclei is seemingly the result of abundant nourishment.

12. First mitotic divisions in the tapetum occur, in general, from the synizetic to the spireme stages of the pollen mother cells. Second divisions take place from spireme stages through the diakinetic periods of meiosis.

13. Irregularities present in nuclear divisions in the tapetum of the plant studied may possibly obtain in other multinucleate tissues, especially those having a similar physiological function.

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## Explanation of plates 19 and 20

Drawings were made with an Abbe camera lucida at table level. A Zeiss 10× ocular, and a Spencer 1.8 mm. oil immersion objective, N. A. 1.3 were used. All drawings are magnified ×700.

### Plate 19

Fig. 1. Uninucleate tapetal cell having a single large nucleolus. Microsporocytes in resting stage at this time.

Fig. 2. A normal first division in telophase and a resultant binucleate cell.

Fig. 3. Tetraploid nucleus due to an incomplete first division.

Fig. 4. Cells with nuclei in a state of pyknosis.

Fig. 5. Final stage in pyknosis.

Fig. 6. Binucleate cell with closely appressed nuclei in late prophase.

Fig. 7. An incomplete mitotic division, and a cell containing a resultant tetraploid nucleus.

Fig. 8. A normal binucleate cell.

Fig. 9. Tetraploid nucleus such as would result from a failure of the separatory process in the first division.

Fig. 10. Tetraploid nucleus with an amitotic appearance.

Figs. 11a, 11b. Dumb-bell-shaped, tetraploid nuclei resulting from incomplete division.

Fig. 11c. U-shaped, tetraploid nucleus resulting from incomplete division.

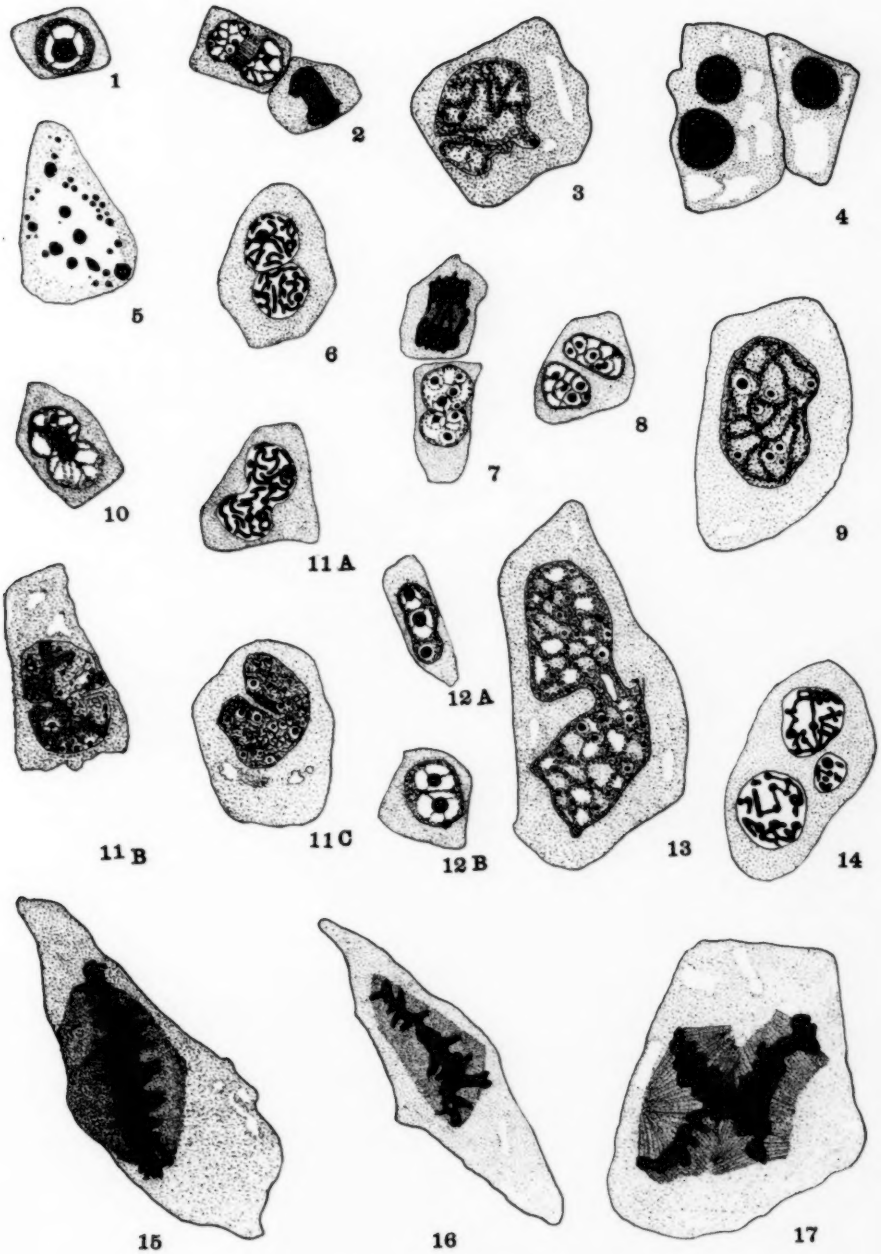
Figs. 12a, 12b. Uninucleate cells during resting stage of microsporocytes. The vesicular condition of the nuclei simulates amitosis or fusion.

Fig. 13. Octoploid nucleus produced by an incomplete second division.

Fig. 14. Trinucleate condition, the small nucleus presumably the result of chromosome isolation during the anaphase of a division.

Figs. 15, 16. A tetraploid nucleus in metaphase, shown in two consecutive sections.

Fig. 17. A highly irregular division with multipolar spindle. This is not a group of three metaphases.

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## Plate 20

Fig. 18. A second division showing two telophase groups fusing due to spindle orientation. Two diploid nuclei and one tetraploid nucleus result.

Fig. 19. A second division showing two telophase groups fusing as in figure 18, but one division being incomplete. One hexaploid, and one diploid nucleus are thus formed.

Fig. 20. Cell having a nuclear complement such as would result from the condition shown in figure 19. The nuclei are presumably hexaploid and diploid.

Fig. 21. Trinucleate cell such as would result from the condition figured in 18.

Fig. 22. Tripolar spindle formed by the division of a tetraploid nucleus.

Fig. 23. Tripolar spindle formed during the first division.

Fig. 24. Non-separation during a second division. Note remnant spindle fibers. Resultant nucleus octoploid.

Fig. 25. Two metaphases of a second division. The first division was normal and gave rise to nuclei that were not appressed.

Fig. 26. Fusion of two telophase groups during a second division.

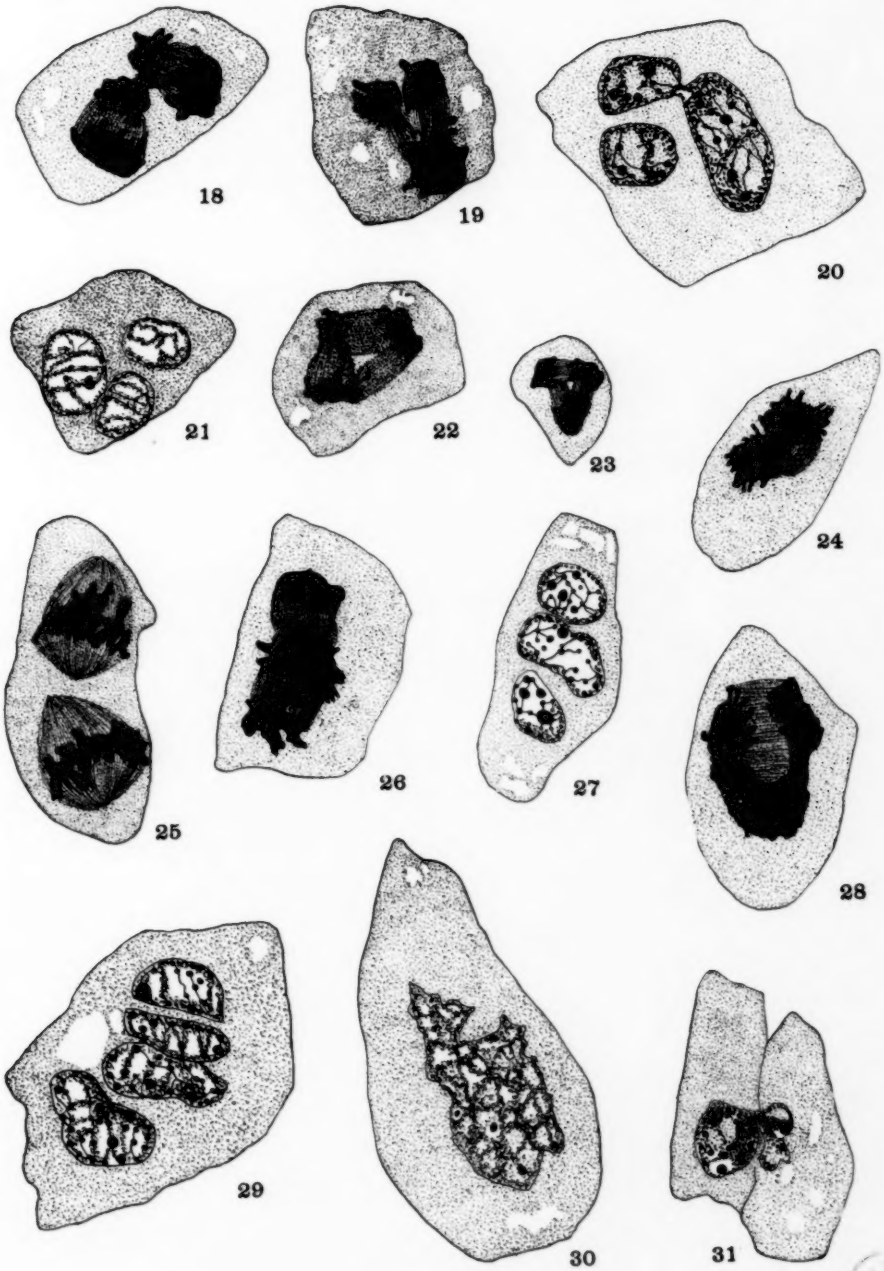
Fig. 27. Trinucleate cell such as might result from the condition in figure 26.

Fig. 28. A second division in which separation was incomplete. A single octoploid nucleus results. Note the spindle fibers.

Fig. 29. Tetranucleate condition resulting from two divisions each of which was complete.

Fig. 30. Cell with octoploid nucleus showing ragged and vacuolate appearance often found in aged tapetal cells.

Fig. 31. Fusion between nuclei of adjacent cells in the aged tapetum.

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## INDEX TO AMERICAN BOTANICAL LITERATURE
### 1931–1935

The aim of this index is to Include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

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IF ANY of the readers of this journal can furnish any information relative to the identity of the following little-known and poorly described "species," such information will be greatly appreciated by Dr. Harold N. Moldenke, New York Botanical Garden, Bronx Park, New York City, who has for some time been engaged in monographing the American *Verbenaceae*. It is very probable that many of the "species" here enumerated do not belong in the genera in which they have been described, but in each case the original descriptions are so incomplete that their true identity cannot be ascertained readily therefrom. Dr. Moldenke would also appreciate learning where authentic herbarium material of these "species" may be found, since he has thus far not discovered any among the thousands of specimens received by him from 46 of the world's larger herbaria in the preparation of his monographs. It may be that some of these "species" have already been claimed by specialists in other groups who had access to authentic material, and if this is the case, Dr. Moldenke would greatly appreciate this information.

(1) *Aegiphila aurea* Turcz., Bull. Soc. Imp. Nat. Mosc. **36**[2]: 218. 1863 [based on J. J. Linden no. 131, from Cuba].

(2) *Aegiphila inflexa* Vell. Fl. Flum. **38** (1825), Icon. **1**: 96. 1827 [from Rio de Janeiro, Brazil].

(3) *Aegiphila macrophylla* H.B.K. Nov. Gen. & Sp. Pl. **2**: 251. 1817 [based on Herb. Willdenow 2831, collected by Humboldt & Bonpland in Venezuela].

(4) *Aegiphila punctata* Turcz., Bull. Soc. Imp. Nat. Mosc. **36**[2]: 219. 1863 [based on a Graham specimen from Jamaica].

(5) *Aegiphila stipulata* Vell. Fl. Flum. **37** (1825), Icon. **1**: 90. 1827 [from Rio de Janeiro, Brazil].

(6) *Aegiphila umbellata* Vell. Fl. Flum. **37** (1825), Icon. **1**: 89. 1827 [from Rio de Janeiro, Brazil].

(7) *Aegiphila virgata* Turcz., Bull. Soc. Imp. Nat. Mosc. **36**[2]: 220. 1863 [based on a collection of J. Miers from Rio de Janeiro, Brazil].

(8) *Callicarpa reticulata* Sw. Prodr. **31**: 1788 [based on a collection of O. P. Swartz from Jamaica].

(9) *Citharexylum longiflorum* Turcz., Bull. Soc. Imp. Nat. Mosc. **36**[2]: 208. 1863 [based on R. de la Sagra no. 50, from Cuba].

(10) *Citharexylum lycioides* D. Don, Edinb. N. Phil. Journ., Jan.–Mar. 1831, 238. 1831 [from Mexico].

(11) *Citharexylum psilacanthum* Turcz., Bull. Soc. Imp. Nat. Mosc. **36**[2]: 207. 1863.

(12) *Citharexylum racemosum* Sessé & Moc. Pl. N. Hispan., ed. 1, 103. 1887–90 [from Mexico].

(13) *Citharexylum reticulatum* H.B.K. Nov. Gen. & Sp. Pl. **2**: 257. 1817 [based on a specimen collected by Bonpland at Gonzanama, Peru].

(14) *Scleroon oleinum* Benth. ex Lindl. Bot. Reg. 29, Misc. 65. 1843; *Petitia oleina* (Benth.) Benth. & Hook. f. ex Hemsl. Biol. Cent. Am. Bot. **2**: 539. 1881–82 [based on a cultivated specimen grown from seeds sent by Hartweg from Mexico].